
National Institute of Neurological
Disorders and Stroke

Intramural Research



Annual Report
Fiscal Year 1992

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES
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National Institute of Neurological
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NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

FY 92 FTE's (Perm Scientists) Non-FTE's*
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****Will be Proposed for Reorganization with Stroke Branch**

***Will be Proposed for Reorganization**

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DIVISION OF INTRAMURAL RESEARCH

National Institute of Neurological Disorders and Stroke
October 1, 1991 through September 30, 1992

Irwin J. Kopin, M.D., Scientific Director

The Division of Intramural Research (DIR), National Institute of Neurological Disorders and Stroke, conducts investigations covering a spectrum of disciplines related to the clinical and basic neurological sciences and integrative neurobiology. The DIR comprises the Office of the Director, the Clinical Neurosciences Program (CNP) and the Basic Neurosciences Program (BNP).

Within the Office of the Director are the Animal Health and Care Section (AHCS) and the Coordinator for AIDS research. The CNP consists of ten Branches and the BNP contains ten Laboratories as well as the Instrumentation and Computer Section and the Neural Systems Section. The CNP is located mainly in the Clinical Center where there are inpatient facilities, a new day hospital area and outpatient clinics together with supporting laboratories. The Neuroepidemiology and Biometry Branches are housed in the Federal Building in Bethesda. The BNP is accommodated mainly in Building 36 with some laboratories in the Park Building in Rockville, and at Fort Detrick in Frederick, Maryland. Research requiring marine animals is conducted during the summer in facilities rented from the Marine Biological Laboratory in Woods Hole, Massachusetts.

Federal Government scientists, support staff, guest researchers and volunteer workers continue to contribute new discoveries which have significant impact on the explosive growth of knowledge about the nervous system and its disorders. Research is initiated by investigators with interests ranging from the fundamental basis for molecular interactions regulating growth, development, function, and pharmacology of the nervous system to clinical development of new diagnostic procedures for early diagnosis, and new therapies for prevention, retardation, cure, or symptomatic control of neurologic disorders. The results of these investigations advance biomedical knowledge and directly or indirectly contribute to the detection, prevention or treatment of nervous system disease or injury, the main mission of the Institute and the NIH. The important accomplishments and status of potentially major advances toward understanding neuronal function and dysfunction are summarized in the Laboratory/Branch reports and in the investigator-initiated research summaries included in the FY 1992 Annual Report. This portion will address those issues having a major impact on the administration and resources (i.e., personnel, available space, and financing) of the Institute.

Management of the DIR is a combined effort involving the active participation of Dr. Mark Hallett, the Director of the CNP (who is also Clinical Director, NINDS), Dr. Harold Gainer, Director of the BNP, and the Scientific Director. During the first two-thirds of this year, Dr. Dale McFarlin served as Acting Director, BNP, while Dr. Hallett was on a sabbatical in his own laboratory. The administrative skills, scientific expertise, wise counsel of the Program Directors, along with able administrative officers, have greatly aided meeting new demands for redirection of resources to ensure optimal utilization of available space, personnel and funds.

New initiatives resulting from recent discoveries and advances in technology, responses to new needs, changes in staffing, and limited availability of space makes necessary continual reassessment of program goals and progress. Optimal utilization of available resources requires planning of appropriate evolution of allocations while ensuring continued flourishing of both clinical and fundamental research.

Personnel

As in previous years, the DIR has utilized fully its employment ceiling. Young investigators, while providing depth of expertise for future roles and expansion of research leadership in new high-priority research efforts, also are particularly important because the disparity in salaries between government and academic institutions or industry makes it extremely difficult to attract senior established investigators to the NIH. It is also difficult to retain promising young investigators; during FY 1992 there have been five tenure actions, David Sibley, Ph.D. (ETB); Maral Mouridian, M.D. (ETB); Barbara Karp, M.D. (OCD); Susan Wray, Ph.D. (LNC); and Michael O'Donovan, Ph.D. (LNLC). NINDS has been fortunate in recruiting from M.I.T., Dr. Ron McKay, who has been appointed Chief, Laboratory of Molecular Biology; he is to begin in this capacity on January 1, 1993.

As is usual for excellent research programs, investigators receive attractive offers to serve in private research organizations or at universities, and during FY 1992, several of our staff have elected to accept such offers. Dr. Ronald Polinsky, Chief, Clinical Pharmacology Section, CNB is now with Wyeth; Dr. Mark Brann left for the University of Vermont; Dr. James Battey accepted a position in the National Cancer Institute; Dr. Krys Bankiewicz has joined Somatix, a new biotechnology company in California; and Dr. Craig Venter, who had been Chief, Section for Receptor Biochemistry and Molecular Biology, has left to head a new research institute supported by private sources. While some of these relocations must be considered losses for NINDS, they also create new opportunities for development of new initiatives. The Central DNA Sequencing Facility which was under the supervision of Dr. Venter will be continued under the aegis of Dr. Lev Goldfarb; and a new Center for Neurogenetics, which will include investigators in both the Laboratories and Branches, has been organized under the leadership of Drs. Goldfarb and Lynn Hudson; as had been planned, this facility will be moved from the Park Building to Building 49 and will assist NINDS investigators in preparing and sequencing oligonucleotides relevant to their neurogenetic research. We hope to have available sufficient space to allow recruitment of an established junior investigator to form a core neurogenetics group. The individual selected is expected to have a strong developmental neurobiology background.

During most of FY 1992, there were over 609 employees utilizing the 518 full-time equivalent (FTE) positions. The personnel included: 169 professional non-tenured FTE employees: 26 clinical associates; 9 staff fellows; 41 senior staff fellows; 16 special experts; and 76 visiting associates/visiting scientists. There were also 85 FTE ceiling-exempt scientists (Visiting Fellow and IRTA positions; National Research Council Fellows), as well as an equal number of Guest Workers, volunteers, and IPA appointments.

Space

Space limitations on the NIH campus have required that some of the NINDS scientists work in rented off-campus facilities. Even after the move to Building 49, off-campus

laboratory space will be required and it is expected that there will be continued occupancy of some space in the Park Building for a limited period; it is hoped that by the end of FY 1994, space in the Park Building can be abandoned.

To meet AALAC accreditation standards, all animal care facilities have been centralized and are under the aegis of the AHCS of the NIH. All animals for NINDS research in Building 36 (except primates with immunodeficient virus infections, primates in LNLC, and a small colony of HSV-infected mice) are housed in the combined animal facility on the mezzanine level of Building 36. Building 10A has become available to house animals in Building 10. Scientists of the Surgical Neurology Branch who are now working in Building 9 and the primates in building 14 will move to Building 49 during FY 1993. Renovations of Nursing Unit 5E were completed during FY 92 and NINDS is awaiting renovations which will provide a new conference room in the solarium area between the nursing units.

Fiscal Issues

At present, it appears unlikely that the level of support for NINDS "other objects" will be maintained; cost of management fund and personnel costs have escalated beyond the expected small increment in total NINDS funding for direct operations. Attempts will be made to preserve high-priority research work on new therapies for brain tumors, gene therapy, and neuroimaging, but it is clear that some adjustments in research efforts will be necessary, new equipment purchases may not be possible and some renovations canceled.

Cooperation with Industry

Scientists in NINDS have initiated Cooperative Research and Development Agreements (CRADAs) with industrial organizations with the goal of commercializing products developed within their laboratories. CRADAs currently active are: (a) Dr. James Battey; Triton Biosciences; Dr. Richard Feldman; Isolation of cDNA that Encode the Murine Gastrin Releasing Peptide Receptor (mGRP-R); (b) Dr. Richard Youle; Haflund Nycomed; Dr. Tore Tsjaberg; Immunotoxins for Central Nervous System Disease; and (c) Dr. Richard J. Youle; Biogen, Inc.; Dr. Roy Lobb; Angiogenen Immunotoxins. These are in addition to several other active CRADAs.

Annual Report: October 1, 1991 to September 30, 1992

Office of the Director

Office of the Clinical Director

Clinical Neuroscience Program, DIR

Mark Hallett, M.D., Clinical Director

The Office of the Clinical Director handles administrative matters, mainly relating to patient care, coordination of educational activities, and delivery of neurologic services. Service functions can be divided into the EEG Laboratory, the EMG Laboratory, the Consultation Service for Neurology, Neuropathology, Neuropsychology Consultation Service and Paraprofessional Support Services.

The two major educational conferences are the Clinical Conference (held on Tuesday afternoon), which is aimed at the Clinical Associates (MSF) and typically reviews a patient in detail, and the NINDS Grand Rounds (held on Friday afternoon). The Clinical Conference includes some attention to matters of patient care and quality assurance. The Grand Rounds continues to offer CME credit.

EEG Section, Susumu Sato, M.D., Chief

Diagnostic Services:

During this reporting period, the laboratory has been staffed with three full-time technologists and has been formulating plans for performing sleep monitoring and outpatient video-EEG monitoring for epileptic patients. The overall EEG and evoked response testing activities slightly increased during this reporting period. There were no significant shifts in the request orders from different institutes, but there was a significant increase in special procedures, the purposes of which were mainly assisting research activities of the NINDS or other institutes. They included preparations for positron emission tomography, intracarotid sodium amobarbital injection, closely spaced EEG recording, intraoperative monitoring, and protocols of event-related evoked potentials, research on somatosensory evoked responses, and others.

	EEG	Evoked Potentials
NINDS	116	63
NINDS OPD	180	32
NIADDK	2	4
NICHD	59	53
NIAAA	25	
NIMH	76	
NIAMS	3	
NCI	13	4
NHLB	26	
NIAID	5	1
NIA	89	
OTHER OPD	180	67
Special procedures	136	
Nocturnal sleep recording	6	
TOTAL	916	224

Participation in Research Activity:

The EEG Section collaborates closely with the Clinical Epilepsy Section of the Epilepsy Research Branch and plays an important role in evaluating epileptic patients. The Section staff monitors and interprets EEGs during sodium amobarbital intracarotid injection, during surgery for the treatment of epilepsy (electrocorticography), and during chronic subdural recording in epileptic patients. The Section staff assists in applying electrodes for video-EEG telemetry recording, magnetoencephalographic study, and neuropsychologic investigation.

Collaborative arrangements have been made for monitoring the EEG and evoked responses in patients with Lennox-Gastaut syndrome, cystinosis, Gaucher's disease, and Menke's disease. The Section will undertake some experimental activity involving nocturnal polysomnographic recording, intensive EEG monitoring of epileptic patients, intraoperative evoked potentials, and computer analysis of epileptiform discharges.

The EEG Laboratory provides a training environment for Clinical Associates toward certification by the American Board of Clinical Neurophysiology and the Added Qualification in Clinical Neurophysiology from the American Board of Psychiatry and Neurology. The Laboratory Chief continues to serve as Associate Examiner on the American Board of Clinical Neurophysiology.

Diagnostic Services:

EMG Laboratory, Carlos Luciano, M.D., Acting Chief

EMG ACTIVITIES (July 1, 1991-June 30,1992)

Number of Examinations	NINDS	198
	NCI	108
	NIAID	27
	NIAMS	25
	OTHER INSTITUTES	14
Normal Volunteers		7

Total	379
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As in the past, a large portion of the studies performed in the laboratory are in patients under the care of the Clinical Center, requested for diagnostic or prognostic purposes. Approximately half of the patient referrals to the EMG Laboratory during the year originated within NINDS, and the other half were requested from other Institutes. Another portion of the studies are from projects in collaboration with other institutes or initiated from within the laboratory.

Participation in Research Activity:

1) Collaborative Studies with the National Cancer Institute

a. The section continues to monitor HIV-positive patients, including AIDS and ARC patients, for signs of neuropathy during treatment with experimental drug regimes. We are currently part of a study in HIV patients with non-Hodgkin's lymphoma receiving infusional chemotherapy, including potentially neurotoxic agents such as ddI and vincristine.

b. We have continued evaluating the effects of taxol, an antineoplastic agent, on peripheral nerves. More recently we have agreed to collaborate in a Phase I study using taxol and cisplatinum in previously untreated ovarian carcinoma patients.

2) Collaborative Program with the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

We have continued studying patients with inflammatory myopathy who are referred for special treatment with steroids, immunosuppression or plamapheresis.

3) Studies on Patients with Nephropathic Cystinosis (Collaborative Studies with the National Institute of Child Health and Human Development)

We have now studied a significant number of patients with this rare disease who have develop hand weakness; we have shown that this is due to muscle involvement rather than nerve damage. We are in the process of submitting our results and description of this previously undescribed complication.

4) Evaluation of Neuromuscular Disease (NINDS Study #84-N-203; Principal Investigator, Dr. Mark Hallett). Studies under this protocol include:

- a. The study of possible peripheral nerve involvement in inclusion body myositis. This disorder primarily affects muscle but recent studies have raised the possibility that the peripheral nervous system (PNS) is also involved. We are prospectively studying a group of affected patients with different neurophysiologic techniques in an effort to explore this possibility.
- b. The use of power spectrum analysis with surface EMG in patients with primary muscle disorders, neurogenic and healthy volunteers. We are studying the diagnostic value of comparing different frequencies in the power spectrum in differentiating myopathies from neurogenic disorders. The use of surface recording will provide a nonpainful alternative to the current needle EMG examination.
- c. The determination of normal values for the minimal latency of F waves recorded from proximal muscles of healthy subjects. We are comparing a new digital subtraction method to other, more cumbersome, currently accepted methods. This will provide an easier way of assessing less accessible parts of the PNS.
- d. The comparison of different methods of stimulation of thPNS, including magnetic stimulation, and the determination of the most likely site of activation when stimulating over the cervical spine.
- e. The comparison of electrophysiologic findings in paraproteinemic and nonparaproteinemic demyelinating polyneuropathies. Preliminary results have shown that sensory fibers are more severely affected in the former, suggesting different immunopathogenic mechanisms.
- f. Quantitative EMG in patients with dermatomyositis without clinical weakness. In collaboration with Dr. Marinos Dalakas we are correlating the electrophysiologic findings with the histologic features in this atypical group of patients with this disorder which typically causes muscle inflammation and weakness.

Neurology Consultation Service, Barbara Illowsky Karp, M.D., Chief

The Neurology Consultation Service consists of two neurologists: Drs. Barbara Karp (Chief), and Eric Wassermann. The service provides emergency and routine neurologic consultation to both inpatients and outpatients throughout the Clinical Center. The consultation services include initial evaluation, facilitation of procedures and testing in other departments (such as neuroradiology and electrodiagnostic studies), and follow-up neurologic care. The Consultation Service arranges for weekly clinical case conferences. The service also provides for education of residents from the National Naval Medical Center who rotate through NIH during their first year of residency.

Patients referred to the consultation service over the last year include:

NCI	198
NIAID	71
NHLBI	53
NIDDK	38
NIMH	29
NICHD	16
OTHER INSTITUTES	44

Total	466
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Ten percent of patients seen by the neurology consultation were HIV-positive.

Participation in Research Activity:

The Consultation Service staff participates in research activities within NINDS and in collaboration with other institutes. Studies being pursued include those under protocols 84N-196, 87N-110 and 85N-195 on human motor control, on neurologic abnormalities in schizophrenia, and on the use of botulinum toxin injection for focal dystonia. Neurologic aspects of AIDS, cancer therapy and pediatric schizophrenia are being studied under protocols in NIAID, NCI, and NIMH.

Publications:

Alexander RC, Karp BI, Thompson S, Khot V, Kirch DG: A double-blind, placebo-controlled trial of demeclocycline treatment of polydipsia-hyponatremia in chronically psychotic patients. *Biol Psychiatry* 30(4):417-20, 1991.

Burt RK, Sharfman WH, Karp BI, Wilson W: Mental neuropathy, herald sign of tumor relapse. *Cancer* (in press).

Karp BI, Juliano DM, Berman KF, Weinberger DR: Neurologic outcome of dorsolateral prefrontal leukotomy. *J Neuropsychiatry Clin Neurosci* (in press).

Karp BI, Vieweg WVR: Rapid correction of hyponatremia in psychiatric patients with polydipsia (letter). *Am J Med* 1991;90:408-9.

Neuropathology, David A. Katz, M.D.

As in previous years, diagnostic neuropathology services for NINDS and for all other Institutes, have been provided by Dr. Katz. The Neuropathology Service is integrated with the Autopsy, Surgical Pathology, and Ultrastructural Pathology Sections and residency training program of the Laboratory of Pathology, NCI; a high priority is given to resident teaching. The brain was examined in a high percentage of autopsies performed at NIH in the last year. Many manifested significant primary or secondary neurologic disease, including, dementias and other degenerative diseases; neurologic complications of systemic malignancy; and AIDS, particularly in the pediatric age group. Brain cutting, held weekly, is scheduled to encourage participation by interested physicians and nurses. Relevant neuropathologic findings are presented formally at gross autopsy and mortality conferences. Selected cases are further utilized for neurologic clinical conferences. Surgical specimens include both in-house and submitted materials, for an annual total of approximately 300 cases; intraoperative frozen-section consultations are required in approximately 25 in-house cases per year. Surgical material includes primary and metastatic brain tumors, pituitary tumors, spinal tumors, vascular lesions, brain biopsies, and muscle biopsies.

The Neuropathology Service also functions in a collaborative manner to provide subspecialty expertise in a range of clinicopathologic investigations. Active current collaborations include the following areas: (1) dementia and degenerative disease (NIMH and NIA); (2) experimental therapy for malignant gliomas (NINDS); (3) pituitary adenomas (NICHD, NIDDK, and NINDS); (4) basic and clinical MRI correlations (NINDS); (5) HTLV-1 myelopathy (NCI).

Publications

Oldfield E, Doppman JL, Nieman LJ, Chrousos GP, Miller DL, Katz DA, Cutler GB, Jr., Loriaux DL: Bilateral inferior petrosal sinus sampling with and without corticotropin releasing hormone for the differential diagnosis of Cushing's syndrome. *N Engl J Med* 1991;325:897-905.

Poirier MC, Reed E, Litterst CL, Katz DA, Gupta-Burt S: Persistence of platinum-amine-DNA adducts in gonads and kidneys of rats and multiple tissues from cancer patients. *Cancer Res* 1992;52:149-53.

ANNUAL REPORT
October 1, 1991 through September 30, 1992

Clinical Neuropsychology Unit
Paul Fedio, Ph.D., Office of the Clinical Director
National Institute of Neurological Disorders and Stroke

Injury to the temporal lobe and limbic structures alters memory, language, and perceptual functions subserving behavior. The study of patients with left or right temporal epileptogenic lesions provides a unique opportunity to examine both cognitive and emotional functions with a wide range of procedures. Our approach combines results obtained by several neuropsychologic techniques, each designed to generate specific information about the organization of brain functions and processes. For example, we examine memory disorders in patients before and after temporal lobectomy with standard and experimental tests and procedures. The results are supplemented by data culled from other techniques, including brain stimulation, neuroimaging with positron emission tomography (PET) and high resolution magnetic resonance imaging (MRI), and the intracarotid amytal procedure (Wada). Each contributes specific and convergent information and produces more globally coherent knowledge about brain functions which no single technique can alone provide. The results provide a basis to formulate theoretical models about the organization of cognitive and emotional functions in the brain and the effects of injury.

In the domain of memory and language, clinical and experimental data are generated during electrical brain stimulation, the Wada procedure, and PET activation with neurologic patients, many of whom present with temporal lobe epilepsy. Different types of memory processes are studied in normal and amnesic individuals, using a variety of experimental and neuropsychologic tests, supplemented with PET and MRI measures of hemispheric specialization. Other study topics and interests extend to anomia, semantic activation, and hypermnnesia.

Using PET activation procedures to identify brain mechanisms responsible for storage and retrieval of information in short- and long-term memory registers, Dr. Fedio and Ms. August identified activation in the left inferior and lateral temporal cortex when subjects performed both memory tasks, more so while learning newly minted material. Left frontal regions were hypermetabolic when the patients selected words (recognition) that they memorized immediately (short-term) or several days before (long-term). There was frontal activation during recall from long-term memory and much less during short-term recall which in turn provoked left parietal and right frontal responsivity. The data indicate that frontal mechanisms are important to recognition memory, whereas the inferiorolateral temporal cortex and parietal lobe are engaged when learning and recall tap into short-term memory stores.

Various encoding strategies were adapted in verbal learning and recall by normals and patients after left (LTL) or right (RTL) temporal lobectomy. The subjects used different mnemotechniques and generated cues to facilitate memory: phonemic [produce rhyme to target item]; spatial [specify location of item]; praxic [show use of item]; and free recall [no encoding]. Recall proficiency was related to extent of hippocampal resection (small [pes only]; large [beyond pes]).

There was no punctiform relation between measures of hippocampal removal and learning impairment. Phonemic cuing produced the largest interference during acquisition and LTL patients did more poorly than RTL and normal subjects even though RTL patients had larger lateral and mesial resections. LTL performance with phonemic encoding was not affected by the size of their resection. Free recall (no encoding) was poorest overall, more so by left temporal patients with large mesial removals. Praxic and spatial encoding was highly beneficial during learning but correlated weakly with resection. These data suggest that the anterior and lateral temporal neocortex is critical for phonologic encoding whereas the semantic memory system requires cortical networks beyond the temporal lobe and most likely involves parietal association cortex.

In a separate series of studies developed by Drs. Fedio, Sato and Kufta, and Ms. August, tests of object naming and working memory were developed and used to identify critical language zones during brain stimulation of indwelling electrodes for the purpose of guiding intended resections. There was special interest to functionally map the basal temporal zones which are inaccessible with standard intraoperative stimulation procedures. Stimulation of Broca's zone produced speech arrest; in Wernicke's area, anomia was elicited and was followed by amnesia for those items that were not named during stimulation. In contrast, paraphasic and anomic errors were evoked with low current stimulation of basal temporal sites in a small number of patients. The common feature of these patients was interictal dysnomia, confirmed by neuropsychometric tests. Finally, this work assumes special diagnostic significance for select patients who are at risk to become dysphasic following standard resections that encroach 4 to 6 cm along the inferior temporal gyrus. These preliminary data extend the findings of Luders et al., that the basal temporal region may mediate language in select patients with mesial and/or inferior temporal epileptogenic lesions. That some patients were anomic prior to surgery supports an interpretation that some language skills may be displaced to systems that manage word retrieval and access to semantic categories.

Mesial and ventromedial temporal structures contribute to the acquisition, rather than long-term retention of memories. Injury to the hippocampus and parahippocampal gyrus produces an amnesic disorder. While stimulating the ventromedial and lateral temporal regions, patients routinely remembered the anomic event, but it was only with fusiform stimulation that they recalled the objects they had misnamed or failed to name during stimulation. Anterograde (storage) and retrograde (retrieval) errors accompanied lateral temporal and temporoparietal cortical stimulation, respectively; stimulation of fusiform sites did not violate memory.

Parahippocampal stimulation produced no anomia but instead, yielded anterograde memory losses. The parahippocampus projects richly to the association cortices and may be an intermediary register where incoming memory traces (immediate/episodic) from the hippocampus interact with neocortical memory stores (long-term/semantic). This explains why new learning is impaired by damage to the hippocampus and parahippocampal gyrus while established semantic memories are relatively spared.

Members of the Unit are developing procedures that may differentiate cognitive operations of the mesial and lateral cortex of the temporal lobe. This includes a collaborative project with Dr. Marianne Regard, University Hospital, Zurich, who has agreed to study patients following amygdala-hippocampectomy with memory test procedures that will be given to patients at NIH following the traditional temporal lobectomy (lateral and mesial resection). The joint project will enable the investigators to contrast the effects of lateral and mesial lesions on cognitive and as well, emotional changes in epilepsy patients.

The diagnostic and research utility of the standard intracarotid sodium amytal procedure (IAP) is influenced by the use of large dosages (125-150 mg) which produces somnolence and disorientation. The untoward effects may account for divergent reports and misclassification of patients with mixed language dominance or at risk for post-temporal lobectomy amnesia. In an effort to temper the global effects, Dr. Fedio adjusted the dosage of amytal to lower levels (75-100 mg) and at the same time developed new and paced tests. Research questions examined how the left and right brain deal with language processes, using semantic, phonologic, spatial, and color attributes in perceiving and remembering objects and words.

The initial analysis reveals parallel processing of form and color attributes are mediated by divergent systems: category knowledge and reading processes rely on well-established semantic and phonologic rules governed by the left brain, and spatial rules by the right brain. Violations of these operational principles render the stimulus and incoming information as ecologically invalid to the respective processing hemisphere, and yield faulty processing.

Another investigation describes what we believe to be the first study of a bilingual patient with multiple neuropsychologic procedures to identify brain mechanisms servicing primary and secondary languages. The study applied psychometric and IAP testing, electrical cortical stimulation via indwelling electrodes, and PET activation studies while the patient performed tasks in both her primary (Spanish) and secondary (English) languages. After the left amytal injection, object naming in Spanish recovered more quickly while reading in both languages was equally impaired. Anomia was elicited with English tasks by stimulation over a large cortical area in the posterior temporoparietal region which also enveloped a focal zone where anomia was evoked in both Spanish and English. Tasks in English activated more brain regions, bilaterally, with greater mesial temporal, parietal, and frontal involvement. The results suggest that in bilingualism, the secondary language is more dependent than the primary language on divergent neural and cognitive processes of both hemispheres.

In sum, the left hemisphere remains dominant for single or multiple languages but the localization of each language differs. While the native language is more focally represented within classic language areas, secondary languages are more diffusely mediated, albeit within the same region; the right hemisphere is more actively involved with second acquired languages. What remains to be studied is the neural network of language development in relation to age at acquisition.

NEURAL BASIS OF EMOTIONALITY

In a separate line of research, organic psychosyndromes and deficits in arousal are being studied by Drs. Davidson and Fedio, and Ms. Ryan. This approach supplements psychometric test data with psychophysiologic indices and examines changes in personality and autonomic responsivity in epilepsy patients. The primary questions examine the contributions of lateral and mesial temporal structures to perception and expression of emotions and how brain structures interplay cognitive as well as emotional processes.

Disparate emotional changes in neurologic patients postulate different brain mechanisms for the emergence of depression or euphoria after left or right brain injury, respectively. Briefly, the right hemisphere assumes a dominant role in regulating and dealing with emotional perception/expression. Denial and imperception follow right brain injury whereas depression and catastrophic reactions accompany left brain lesions.

Patients with temporal lobe epilepsy (TLE) afford an exciting opportunity to probe limbic mechanisms and the effects of epilepsy on personality development. Ms. Ryan secured clinical profiles of TLE patients and showed that those with right TLE endorsed a positive self-image versus the negative image cast by left TLE patients who also admitted greater maladjustment and personal defects, and poor social relationships. Self-ratings by right TLE patients were much less harsh and critical, and emphasized self-assurance. Left TLE patients endorsed dependency, schizoid and avoidant behavior, heightened anxiety and somatic preoccupation, whereas the profile of right TLE patients was more normal, albeit contaminated by histrionic and narcissistic features.

Temporal lobe lesions invading the amygdala and adjacent mesial temporal structures diminish emotional arousal and reactions. The asymmetry in the alerting capacity of the left and right brain predicts that right brain injury is more likely to produce inattention and hypoarousal. This model was tested by Drs. Davidson and Fedio who monitored effector limbic activity via electrodermal (EDR) measures in patients after unilateral temporal lobectomy. Skin conductance levels (SCL) and responses (SCR) were recorded while TLE patients performed habituation and discrimination tasks. All normal and lobectomy subjects demonstrated diminished responsivity and habituation, but left TLE patients showed the slowest rate of habituation, or conversely, the highest level of arousal. Right TLE patients tended to extinguish SCRs very rapidly and normal subjects assumed an intermediate rate of change. Left TLE patients also showed higher rates of nonspecific fluctuations (NSF) which is concordant with clinical evidence of their increased arousal and anxiety.

The rapid extinction by right TLE patients persisted and later playback of the same tone during the subsequent discrimination condition was not evocative. Unilateral temporal lobectomy, including the amygdala and adjacent limbic structures did not produce hypoarousal, per se. Decreased electrodermal responsivity was evident only for right TLE patients who had relatively intact EDR resting levels. When provoked, however, these patients showed extremely

rapid habituation whereas left TLE patients remained overly responsive and hypervigilant. In the realm of theories of emotionality, the data suggest that injury to the left temporal lobe may exacerbate vigilance and anxiety, and produce an overinclusive orientation. In contrast, injury to the right temporal lobe produces inattention and hypoarousal that may be overcome and activated by novelty and trenchant emotional changes.

Spectral analyses of EEG activity (alpha) were derived while subjects rated emotionally evocative sounds, exploring the hypothesis that emotions are better perceived by the right hemisphere; the right and left brain are specialists in negative and positive emotions, respectively. There was greater right brain activation for normal and both TLE groups, primarily frontal. Left TLE patients were more strongly aroused overall and right-lateralized than right TLE and normals. In rating the stimuli, left TLE patients displayed a negative bias while right TLE patients made errors in positive and negative categories. Left TLE patients evinced significantly higher levels of EEG arousal, and a negative affective outlook. Left temporal lesions may deactivate inhibition and amplify the emotional state of the right brain, symptomatically expressed as tonic arousal and anxiety. These data provide valuable insight into the mechanisms of phobia, panic and stress disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01658-25 OCD*

PERIOD COVERED

October 1, 1991 through September 30, 1992**

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hemispheric Development and Specialization of the Intellectual Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Fedio, Ph.D.	Unit Chief	CNU, OCD, NINDS
	E. Mohr, Ph.D.	Psychologist	Ottawa, Canada
Others:	T. Blaxton, Ph.D.	Senior Staff Fellow	CES, ERB, NINDS
	S. Bookheimer, Ph.D.	IRTA Fellow	CES, ERB, NINDS
	L. Ryan, M.A.	Psychologist	CES, ERB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Clinical Director

SECTION

Clinical Neuropsychology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The effects of chronic progressive neurologic disorders in adults and children were evaluated by a broad range of neuropsychologic tests evaluating brain-behavior relations. A neuropsychologic profile was plotted for patients with Alzheimer's (AD), Huntington's (HD), or Parkinson's (PD) diseases. The evaluations extended into memory, learning and perception, applying standard and experimental tasks to identify functional changes accompanying the aging processes.

The results implicated dopamine deficiencies and frontal pathophysiology in PD, most notably, losses in executive capabilities and visuospatial and generic memory functions. With HD patients, perceptuomotor capacity and the ability to manipulate spatial information were affected whereas spatial discrimination was relatively intact. With a dichotic task, AD patients did poorer and were unable to selectively attend to serial information. The behavioral data extend neuropathologic impressions of degeneration of the frontal striatal system in HD and temporoparietal, cortical involvement in AD.

**THIS PROJECT WAS TERMINATED 10/91 start of new FY.

*Formerly in MNB; transferred to OCD in 9/91.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

701 NS 01424-26 OCD*

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Modulation by the Limbic System in Man

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Fedio, Ph.D.	Unit Chief	CNU, OCD, NINDS
Others:	R. Davidson, Ph.D.	IRTA Fellow	CNU, OCD, NINDS
	L. Ryan, M.A.	Psychologist	CES, MNB, NINDS
	A. August, M.A.	Psychologist	CNU, OCD, NINDS
	C. Kufta, M.D.	Medical Officer	SNB, NINDS
	S. Sato, M.D.	Medical Officer	EEG, OCD, NINDS
	W. Theodore, M.D.	Medical Officer	CES, ERB, NINDS

COOPERATING UNITS (if any)

Surgical Neurology Branch, DIR, NINDS
Clinical Epilepsy Branch, DIR, NINDS

LAB/BRANCH

Office of the Clinical Director

SECTION

Clinical Neuropsychology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Personality and mood characteristics were studied in epileptic patients before and after unilateral left or right temporal lobe resection or intracarotid amytal injection. Physiologic events (skin conductance) and EEG were also monitored during evocative procedures. The research examined the role of the left and temporal lobes in emotional perception and expression, and how brain injury alters these functions.

Skin conductance responsivity was recorded from patients following unilateral left or right temporal lobectomy, and normal subjects. Right temporal lobectomy (RTL) patients showed rapid habituation and extinction whereas left temporal lobectomy (LTL) patients showed increased arousal and hypervigilance. In a behavioral paradigm, RTL patients were more responsive to failure than LTL patients.

LTL patients demonstrated anxiety, fearfulness and avoidant features; RTL patients presented a dramatic more emotional style. This was confirmed with intracarotid amytal where euphoria followed right and dysphoria followed left injections.

*Formerly in MNB; transferred to OCD in 9/91.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01245-27 OCD*

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
EEG Learning Correlates Using Scalp and Intracranial Depth Electrodes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. Fedio, Ph.D.	Unit Chief	CNU, OCD NINDS
Others: S. Sato, M.D.	Medical Officer	EEG, OCD, NINDS
A. August, M.A.	Psychologist	CNU, OCD, NINDS
C. Kufta, M.D.	Medical Officer	SNB, NINDS

COOPERATING UNITS (if any)

Surgical Neurology Branch, DIR, NINDS

LABORATORY
Office of the Clinical Director

SECTION
Clinical Neuropsychology Unit

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS 1.0

PROFESSIONAL: 0.5

OTHER 0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Cognitive and emotional processes were monitored during brain stimulation and with electroencephalographic (EEG) activity from indwelling electrodes in patients with temporal lobe epilepsy (LTE), relating left and right brain dysfunctions to maladaptive ideative and emotional reactions, respectively.

Electrical brain stimulation identified a unique group of patients with language functions in the left basolateral temporal lobe, outside the classic brain zones. These patients are anomic interictally and at-risk for postoperative dysphasia.

In an EEG study, LTE patients were more strongly aroused by evocative stimuli and had a more negative perceptual bias than right temporal patients. These data underscore the dual cognitive and emotional roles of the limbic system in modulating human behavior.

*Formerly in MNB; transferred to OCD in 9/91.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 00200-38 OCD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cognitive and Emotional Profile of Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Fedio, Ph.D.	Unit Chief	CNU, OCD, NINDS
Others:	L. Ryan, M.A.	Psychologist	CNS, ERB, NINDS
	D. Ronsaville, Ph.D.	Psychologist	CNU, OCD, NINDS
	A. August, M.A.	Psychologist	CNU, OCD, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Clinical Director

SECTION

Clinical Neuropsychology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experiments using brain stimulation, PET imaging and behavioral procedures were initiated to identify the neuroanatomic basis underlying different types of memory and language disorders exhibited by patients with neurologic disorders.

Results from encoding and memory tasks showed that left temporal lobe is critical for phonological processing whereas semantic memory involves mesial as well as lateral temporal-parietal cortex.

Brain stimulation, intracarotid amytal injection and PET imaging in a bilingual patient showed that the left hemisphere assumes a dominant role in primary and secondary languages. The brain representation of the primary language, however, is more focal in the left hemisphere. The second acquired language utilizes a larger cortical region in the left and dominant brain, and as well, the right hemisphere for paralinguistic functions.

*Formerly in MNB; transferred to OCD in 9/91.

ANNUAL REPORT

October 1, 1991 - September 30, 1992

Instrumentation and Computer Section

Office of Director, Basic Neurosciences Program, DIR

National Institute of Neurological Disorders and Stroke

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INSTRUMENTATION AND COMPUTER SECTION

Office of Director, Basic Neurosciences Program, DIR

National Institute of Neurological Disorders and Stroke

October 1, 1991 - September 30, 1992

Bruce M. Smith, Ph.D., Chief

ORGANIZATIONAL STRUCTURE AND SERVICES

The Instrumentation and Computer Section provides technical support for the intramural staff of NINDS and NIMH by: (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation systems; (3) designing and specifying laboratory computer systems for data acquisition and processing; (4) designing and developing custom software for scientific and administrative applications; (5) managing a central computer facility in the Weicker Building consisting of a multiuser MicroVAX 3600, an HP 730 UNIX server, an image processing system, and a network of Macintosh personal computers and LaserWriter printers; and (6) developing and managing networks in the Weicker Building and in the Clinical Center. An additional important service provided by Section personnel is consultation on a wide range of topics in the areas of instrumentation, computer science, mathematics and statistics.

When the services of the Section are requested, the investigator first meets with the Section Chief and other appropriate personnel to discuss the requirements. On the basis of this meeting, a decision is made as to whether the Section will take on the project. If a commercial product will satisfy the requirements, the investigator is advised to purchase it. If a custom design is required, we will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases, the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Program.

When the Section Chief or the Assistant to the Chief agrees to accept a project, a standard Section work request form is initiated. The Section member leading the project then confers with the investigator to formulate a set of specifications and a cost estimate for the project. This information is recorded on the work form which the investigator and his/her Lab Chief sign to authorize the project. The Section does not charge for services; however, upon completion of the project, the investigator's laboratory or branch is billed for the cost of the components used. Reimbursement of funds takes place at the beginning of the next fiscal year.

INSTRUMENTATION

The Section has a staff of five engineers, five computer specialists and four technicians to design and produce special-purpose instrumentation. It is often appropriate for an engineer, a technician, and a computer specialist to work together to combine electronic and/or mechanical hardware, a personal computer or microprocessor, and custom software, to produce cost-effective solutions to instrumentation problems. The following are brief descriptions of the Section's major projects, taken from a total of more than 300 projects undertaken this year.

Ambulatory Patient Activity Monitoring System. The Section has continued to develop the Patient Activity Monitor (PAM) and the hardware and software which form the system. Intramural investigators and their outside collaborators are using the system in their studies and treatment of depression, hyperactivity, schizophrenia, alcoholism, sleep and eating disorders, and animal models of Parkinson's disease. **Monitor:** Two versions of the PAM are now being supported. The older version has a 1K-byte memory and normally accumulates activity values in 15-minute recording intervals. A new device with a 32K-byte memory and selectable recording intervals of 0.5, 1.0 and 2.0 minutes is now ready to be phased into use. Twenty-five of the new monitors have been produced and are now undergoing final checkout and calibration. **Computer Support:** The Section supports a PAM readout system based on the Macintosh personal computer coupled to a microprocessor-controlled serial interface. A comprehensive Macintosh PAM program handles data readout and disk filing, graphic data editing, construction of continuous data files and plots, and formation of tabular data sets for transfer into spreadsheet and statistical applications. The PAM readout program and the serial interface have been redesigned to accommodate the new 32K PAM and its much higher readout speed, while also maintaining compatibility with the older PAM. **IMS CRADA:** In May, 1989 the NIMH and Individual Monitoring Systems, Inc. (IMS) entered into a Cooperative Research and Development Agreement in order to work together to further the development of the PAM technology for the benefit of the NIMH and the general public. By last year, IMS had produced more than 50 units of the 1K PAM, along with the companion interface units, for commercial sales to research and medical markets. IMS and the Section utilized their joint expertise this year to finalize the design and fabrication methods for the 32K PAM and the new readout interface, and arranged to share the first production runs of the new monitor. Efforts are also continuing to extend the PAM technology to monitor other parameters, such as eye blinks from ambulatory subjects.

Controllers for Perfusion Studies. The Section has continued to make significant improvements to two popular perfusion systems that are used to study the effects of extracellular drug concentrations on cell properties. One system uses a linear-actuator stepper motor to rapidly switch the position of a linear array of nine micropipettes. The solution in each barrel of the array is driven at the same flow rate by a multichannel pump and is gated on and off by a nine-channel valve controller. Previously, a microprocessor system was developed to provide the precise timing between the stepper motor movements and the gating of the valve controller. The investigator used a personal computer to select perfusion sequences and to download them to the microprocessor. This year, the computer program was greatly expanded to incorporate all the functions of the microprocessor circuitry. A low-cost input/output timer board was used in the personal computer to

provide the drive pulses to the motor control chip and to the valve controller. The second type of perfusion system requires no pipette movement. A special micropipette holds nine individual solution tubes whose ends all converge very close to the tiny common exit port at the end of the micropipette. The flow of each solution is again controlled by small valves. A second generation controller was developed last year to provide automatic perfusion sequences and low-power valve switching. The versatility of the controller was considerably increased this year by allowing any combination of the nine nonvolatile programmable sequences to be grouped together and run sequentially. Additionally, the controller was expanded to produce outputs and to accept inputs so that its operation could be synchronized to and/or monitored by other equipment, such as data acquisition or image processing systems. Two of these enhanced perfusion controllers were built, one for the nine-tube micropipette described above, and one for a 12-valve system with independent solution tubes.

Perfusion Pump Controller. In order to carryout expanded experimental protocols with the stepper-motor perfusion system described above, it is necessary to wait 30 seconds or more between perfusion sequences. To conserve valuable drug solutions and to avoid cell damage, the speed of the perfusion pump must be reduced automatically during the inactive time. A programmable timer/speed controller was developed to implement the following functions: accept a trigger pulse from the perfusion controller and wait for a preset time for the perfusion sequence to end; decelerate the pump to a slower speed and wait a preset time; and accelerate the pump back to normal speed just before the next sequence begins. The operator sets the speed and timing parameters with pushbuttons and verifies the settings on a LCD display.

Thalamus Interface. Several intramural labs utilize ISA or EISA computers with custom *Cortex* software for data acquisition, control, and analysis of single unit recordings from primates. A multi-function device, called *Thalamus*, has been developed to serve as an interface between the experimental equipment and the computer's analog and digital input/output boards. *Thalamus* provides convenient interconnection and circuitry for pulse capture (for spike inputs), for pulse generation (for reward delivery), for anti-aliasing filtering prior to A/D conversions, and for touch-pad inputs. A prototype version is currently being tested in the research environment. Since up to ten of these interfaces may be required, a set of five printed circuit boards has been designed to facilitate fabrication and to increase reliability.

Adjustable-Height Elevated Plus Maze. This system was developed last year for studies of anti-anxiety drug effects in mice. The plus maze consists of four equally-spaced elevated runways which intersect directly above an adjustable-height central support base. One set of opposing runway arms are open, with only a small lip on the outside edges. The other set of arms are enclosed on the sides and rear with black plastic. The two open and two enclosed arms are separated by the central intersection area. The transitions from the central area to the four arms are monitored with double horizontal sets of infrared emitters and detectors. A microprocessor system tracks and counts the animal's entries into the two types of runways and totals the duration time in each type and in the central intersection. Four of these automated mazes were completed this year. A companion printer system was also developed to sequentially print the test results from all four mazes.

Elevated Roller Treadmill. A automated roller treadmill was developed for normal and convulsive locomotion studies in rats. A cylindrical roller with a four-inch diameter is positioned two feet above the base of the device and is divided by adjustable partitions along its length into individual animal sections. The roller is belt-driven by a DC motor whose acceleration, steady-state speed and running time are selected by the user and stored in the microprocessor-based circuitry.

High-Gain Stereotrode Amplifier. To aid ongoing efforts to obtain simultaneous, but distinct single-unit recordings from two neurons in close proximity, a high-gain amplifier system has been designed for use with a new dual-channel metal microelectrode (stereotrode). The system amplifies each signal by a factor of 10,000 with a bandwidth of 300 to 10K Hz. The difference between the two signals is also amplified and filtered with the same specifications. The battery-powered system was designed with emphasis on low-power, low-noise amplification and a high degree of shielding, signal guarding, and RF-suppression on the inputs. Two similar additional models were also developed: a dual version fabricated in the same small package for use with two stereotrodes, and a single stereotrode system with a low-frequency response extended to 10 Hz.

Composite Video Mixer. This project involved development of an interface between a scanning confocal microscope and a video image processing system. The separate, non-standard video signals from the microscope were conditioned and used to generate a composite video signal compatible with the A/D conversion circuitry of the image processing system. A new integrated circuit which combines a video amplifier with a 4-channel high-speed analog multiplexer was used to implement the composite video signal with proper bandwidth and line-driving capability.

Photon-Counting D/A Interface. Single photon events collected by a photomultiplier tube generate pulses of approximately 100 nanoseconds in duration with an amplitude of 1 mV or greater, and occur at a frequency of 1 MHz or less. An instrument was developed to amplify these events, count them, and generate a proportional 8-bit D/A signal for conversion by a PDP-11 computer. The value of the photon-counting interval is selectable by the user and is synchronized with the computer acquisition system for proper correlation with other acquired signals.

Production of Previously-Designed Instrumentation. Considerable effort was involved in the duplication of instruments and devices that had been previously developed in the Section but were requested this year to satisfy additional needs. The systems that were produced included: 4-channel amplifier/filter systems (2 units); 5-channel variable-frequency pulse generators with gated pulse train capability (3 units); dual-channel sharp-cutoff bandpass filter systems (2 units); a 2-channel variable-frequency pulse generator with high-power biphasic outputs; a 16-channel rat rotometer acquisition system with Macintosh serial interface; a voice-activated switch with computer parallel interface; and a custom two-button reaction time device with computer serial interface. In addition, an automated light-dark activity apparatus developed more than ten years ago for anti-anxiety drug studies was redesigned this year with current technology. Two of these activity systems were produced for use in the animal facility in the Clinical Center.

NIH Scientific Directors' Voting System. Last year following a special request by the NIH Associate Director for Intramural Affairs, the Section designed a new voting system to be used by

the NIH Scientific Directors at their bi-weekly meetings. Fabrication and checkout of the 32 individual voting units, the 7 interconnecting junction boxes, the master control unit, and the storage cart were completed early this year. The system has been in routine use since then helping the Scientific Directors efficiently deal with a wide variety of personnel actions. The system provides a high degree of confidentiality and has proven to be easy to use and reliable.

MACHINE SHOP FACILITY

The Section maintains a well-equipped machine shop which is specialized for working with metals and synthetic materials. This facility is critical to the development and fabrication of the electronic and electromechanical instrumentation projects described above. Two technicians also utilize this facility to independently specify, design, and fabricate a wide range of mechanical devices as part of the Section's efforts to provide a spectrum of services in support of basic and clinical research. These staff members are available to advise investigators on mechanical principles and on the properties and uses of materials. Many investigators and other intramural staff frequently come to the shop for immediate help with a small mechanical problem whose timely solution is crucial to their ongoing research. Trained technicians from other labs use our facility to augment the limited capabilities in their own areas.

The following list illustrates the range of mechanical design and fabrication projects typically provided by our machine shop staff: a wide variety of chambers for biological preparations, including tissue cultures, electrophoresis gels, and static and dynamic temperature-controlled perfusion systems; modifications to micromanipulators and to microscopes and other optical devices; modifications to animal chairs, restraining devices and enclosures; pipette holders and storage racks, including radiation shields and collectors; a variety of Faraday cages and enclosures; and numerous adapters for commercial instrumentation.

Our machine shop milling capabilities were significantly enhanced this year by two interrelated developments. First, one of our vertical milling machines was retrofitted with a Computerized Numerical Control (CNC) for automated milling operations in 2.5 axes. Secondly, we purchased a powerful set of Macintosh-based Computer Aided Design/Computer Aided Manufacturing (CAD/CAM) programs which are used to rapidly generate milling programs that are compatible with the CNC machine. The combination of the CNC mill and the CAD/CAM software has been used very effectively by our staff to carryout faster and more precise setups for milling operations, to implement more sophisticated milling operations, and to rapidly duplicate prototype parts.

COMPUTER SUPPORT

In addition to the development of special instrumentation systems, the Section provides support for laboratory and office computer systems and maintains central computer facilities in the Weicker Building for high-capacity data storage, complex off-line data analysis, image processing,

scientific word processing, and high-quality printing and plotting. These support services are detailed under the following categories.

LABORATORY COMPUTERS

Small minicomputers and personal computers are widely used in the intramural laboratories for real-time data acquisition and control, mathematical and statistical data analysis, graphics, and word processing. The Section provides consultation on the specification and selection of these systems and helps with the procurement, installation and maintenance of the equipment. Training in operating systems, programming languages, networking and maintenance issues is available for scientists or laboratory support personnel. Within manpower limitations, the Section develops custom software for specific applications. Section computer specialists are always available for consultation and will aid the investigator in writing the difficult time and data dependent sections of real-time programs. Section specialists also evaluate commercial software or programs from other research facilities to determine their utility for intramural laboratory systems.

We have selected the Apple Macintosh family of computer systems as our standard for support of scientific applications. The Section has substantial experience using Macintosh computers to provide solutions for low-speed laboratory data acquisition projects. Two years ago, the Section developed comprehensive specifications for a Macintosh II-based system for the acquisition of real-time data and control of laboratory devices at high speeds. An outside contract was awarded and a versatile program called the Neurophysiological Data Acquisition Program (NDAP) was developed. NDAP was designed to be useful in all disciplines acquiring data, either analog or discrete, in a continuous or event-triggered mode. It contains modules for event-centered graphic displays, signal averaging, baseline reference monitoring, voltage clamping, pre-programming experimental paradigms, maintenance of the experimental logbook, interactive experimental control, apparatus control, and high-speed, continuous data acquisition. Thus far, NDAP has been used primarily in several NICHD laboratories, and is available at no cost for use by all NIH employees.

VAX FACILITY

The Section maintains a DEC MicroVAX 3600 for use by all intramural staff. The 3600 system includes 32 megabytes (MB) of RAM, a 622 MB RA82 system disk, four 664 MB removable SCSI user disks, an Emulex 8 mm 2.3 gigabyte (GB) helical scan tape drive, a TK70 296 MB cartridge tape drive, and a TSV05 1600 BPI tape drive to maintain media compatibility with older systems. VAX/VMS 5.4 is currently installed as the operating system. Pascal and FORTRAN compilers are available for program development. Approximately 50 RS-232-C hard-wired cable connections are connected to two Emulex P4000 terminal servers, which access the VAX via DEC's Local Area Transport (LAT) protocol over Ethernet. Users can also gain access at 1200 or 2400 baud on five dial-up lines, or at 9600 baud anywhere on DCRT's NIHnet via the Telnet protocol. VAX user accounts have now increased to more than 200.

VAX system software includes AlisaTalk, a package that provides central network file and printing services via AppleTalk protocols to personal computers on the NIH campus-wide network. The Transmission Control Protocol/Internet Protocol (TCP/IP) networking software provides mail, file transfer and terminal sessions to and from a diverse population of NIH campus computer systems as well as the large number of machines on the Internet world-wide network.

The most popular package on the VAX is the sequence analysis software from the Genetics Computing Group (formerly the University of Wisconsin Genetics Computing Group.) This package includes over 100 programs, extensive documentation, and complete on-line help. The Section provides the complete GenBank, NBRF Nucleic, PIR Protein, EMBL and SwissProt databases, and updates all of them quarterly.

UNIX SERVER

To complement the MicroVAX 3600, the Section has purchased a Hewlett-Packard Series 9000, Model 730 workstation to function as a network server. With approximately 20 times the computing power of the MicroVAX, the HP 730 can handle computational-intensive tasks that now take several hours or more. The HP 730 is equipped with 32 MB of RAM, 1 GB of disk storage, and a 1.3 GB DAT tape drive for backup. An additional 16 MB of RAM and 1.5 GB of disk storage will be added in the near future. This new system is connected to the Ethernet portion of the Section's network and through it to the NIHnet and to the international Internet.

As an alternative to the proprietary VMS operating system used on the MicroVAX, the HP 730 provides the UNIX operating system environment that has become popular at the NIH and in the scientific community in general. UNIX provides better support for the TCP/IP file sharing and electronic mail protocols that have become standard on the NIH campus and throughout the scientific community. The Section has taken advantage of the anonymous File Transfer Protocol (FTP) feature on UNIX systems to make software developed by the Section, such as *NIH Image*, freely available to scientists all over the world. The HP 730 has also been set up to function as a Post Office Protocol mail server to provide electronic mail access to the Internet and Bitnet for personal computer users on the NIHnet.

COMPUTER NETWORKS

The Section has continued to expand its network linking Macintosh, Digital, and IBM-compatible computers via the AppleTalk, DECnet, and TCP/IP protocols. The original LocalTalk and PhoneNet network has evolved into a large internetwork including LocalTalk, PhoneNet, a thickwire Ethernet multiport transceiver, 9 thinwire Ethernet segments and a large unshielded twisted pair (UTP) Ethernet star-wired network. The LocalTalk and PhoneNet networks provide low speed connections suitable for printing services, Telnet terminal emulation, electronic mail and the transfer of small files between machines. Ethernet's much higher speed makes the transfer of larger files practical, and is fast enough to allow applications to be shared on a server machine.

There are now 14 LocalTalk/PhoneNet laboratory networks, including two in the Clinical Center, that are linked to the Ethernet portion of the network through 9 gateways. The Ethernet portion includes the MicroVAX 3600, the HP 730 server, a VAXStation 3200, a Silicon Graphics UNIX workstation, a Sun SparcStation II, three PDP-11s and numerous Macintoshes and IBM-compatible PCs. The Section provides a Macintosh II AppleShare file server and an AlisaShare file server on the MicroVAX 3600. Several labs run servers on their portion of the network, including AppleShare, LANTastic, and Novell NetWare servers, and many users are taking advantage of the 10-user AppleShare server built into the Macintosh System 7.0 software.

The Ethernet UTP portion of the network was implemented in the Weicker Building this year. The initial installation by AT&T included 64 workstation nodes connected to eight 12-port repeaters distributed in the W-17 wiring closets on each of the 5 floors. For redundancy, each closet has 2 UTP connections to the main hub in our central computing facilities on the second floor. Additional nodes and repeaters have been added as needed.

Early this year, the Section participated in a pilot project as the first NIH network to route AppleTalk protocols onto DCRT's NIHnet backbone. Following this successful collaboration, DCRT decided to implement AppleTalk routing throughout the NIH network. Another important development occurred in May of this year when our T1 (1.5 Mbits/sec) link to the campus network was upgraded to Ethernet speeds (10 Mbits/sec).

In addition to these networking facilities and activities centered in the Weicker Building, the Section is involved in a major new effort to implement a PC-based network in the Clinical Center to support the administrative, budget and personnel functions of the NIMH intramural program. An additional computer specialist with the appropriate expertise was recruited to lead this effort. UTP Ethernet wiring and 4 multiport repeaters have been installed to provide connections for 34 IBM-compatible PCs within the Office of the Scientific Director (OD), the Budget Office, the Administrative Area A, and the Personnel Office. The repeaters have been connected together and to the NIHnet by DCRT's fiber-optic repeaters. Following discussions with DCRT, Microsoft LAN Manager was chosen as the network operating system and has been installed on a Gateway 2000 486 33 MHz server machine. LAN Manager has been installed on most of the PCs and their network connections have been established. The computer specialist has developed and installed on the LAN a database management system for document and correspondence tracking within the OD. Commercial single-machine applications now being used for budget and personnel functions are being evaluated for use on the network. Microsoft Mail will be installed in the near future and will be supported by DCRT on the NIHnet backbone.

Future plans for this LAN include establishing connections to Administrative Area B in the Weicker Building and Administrative Area C at St. Elizabeth's Hospital via the NIHnet, and then making at least one connection to each NIMH intramural lab and branch office.

NIH IMAGE AND THE IMAGE PROCESSING FACILITY

The *NIH Image* processing program for the Macintosh, which has been under development by the Section for almost five years, continues to be popular with scientists in the intramural programs and throughout the world. Important new features have been added in the last year: a routine for removing smooth continuous backgrounds from one and two-dimensional electrophoretic gels and other images; a routine to generate animation sequences by projecting a rotating 3D data set onto a plane; a command for creating composite images from the slices in a 3D stack; and the ability to save selection outlines to disk and restore them later. In addition, macro routines were written for reslicing 3D MRI data sets and for doing cell counting.

Included in the Section's central computer facilities in the Weicker Building is an image processing system consisting of a Macintosh IIfx with 20 MB of RAM and a 19-inch color monitor, a video camera and lightbox, and a digital film recorder for the production of presentation quality 35 mm slides. The *NIH Image* program is used to acquire, enhance, analyze and print images. The facility is useful for numerous applications, including analysis of CT, MRI or PET images, receptor binding studies, analysis of electrophoretic gels, and quantitative evaluation of cerebral blood flow, glucose metabolism, or protein synthesis. Because our facility is based on the relatively inexpensive Macintosh personal computer, and is simple to install and maintain, investigators with extensive image analysis requirements can easily duplicate it for use in their own laboratories.

PERSONAL COMPUTER FACILITY

Also included in the Section's central computing facility are one Macintosh Plus computer, three Macintosh II computers, a Shiva NetModem, three LaserWriter printers, an Apple flatbed scanner with optical character recognition software, a La Cie SilverScan color scanner, and a Montage slide maker. A variety of Macintosh software is available for intramural scientists to use for statistical analysis, for communicating with DCRT's mainframes and MEDLINE, and for word processing, including creation of posters, slides, and publication-quality charts and graphs. Virus detection programs on all the machines have been updated to maintain protection from new computer viruses. The machines are periodically checked for software copyright compliance, and any programs left on the hard disks are periodically purged to maintain compliance. Important additions to this facility that were purchased this year are a Tektronix Phaser III Pxi color laser printer, Macintosh IIfx processor upgrades for the three Macintosh II machines, and a broader selection of Macintosh commercial software to more fully cover the diverse requirements of our users.

COLLABORATIVE SUPPORT

Section specialists provide collaborative support for selected research projects within the intramural programs. They provide expertise in computer applications, software development, and statistical analysis and experimental design. These efforts and the resulting software developments are described below.

Extensions to NIH Image. Support for research projects in both NIMH and NINDS has led to the inclusion of routines into the *NIH Image* program to count cells and to analyze the spatial distribution of cells in cultures. In the NIMH studies, the number of cells surviving after pharmacological treatment of a colony is the relevant measure of the effect of that treatment. In NINDS, a direct evaluation of developmental changes in the interactions among cells is required. In both cases, cells are either recognized by appropriate filtering and morphological attribute evaluation or they are manually marked for further analysis. Macro routines count all the cells or count the cells found in randomly-placed rectangles, then produce a list of counts and/or a list of x-y coordinates. In the spatial distribution experiments, further analysis is currently being done using commercial statistical programs. If these methods prove to be of general use, the statistical steps will be added to *NIH Image* as well. In support of a second NIMH study, *NIH Image* is being further extended to control the motorized stage of a microscope to automatically scan a brain section slide. Following a scan, the program will identify and label cells, store their locations and plot an image of the section showing the labelled cells.

Morphological Classification of Cells. In collaboration with two NINDS labs, LNP and LNC, a method of analyzing cell shape has been developed using a Fourier transform of the outline of cells produced by an edge detecting technique. This method is now being applied to studies of neural and glial cell images.

Nonlinear Dynamics in Electrophysiology. The Section is collaborating with Children's Hospital on the application of phase space analysis of the nonlinear dynamics of cells (commonly referred to as chaos theory) as evidenced by transmembrane voltage measurements. Programs originally developed at Bryn Mawr College for the IBM PC were first ported to the Section's MicroVAX. Due to the very long calculation times, these programs are now being ported to the HP 730 workstation. Thus far, studies have involved the analysis of voltage recordings in chick cells exposed to agents which modify lipid metabolism. Two papers are in preparation: one on the methodology and one on the results of the work described above.

Analysis of Rhythmic Phenomena. In collaboration with LCB, NIMH, the Section has begun to develop new methods to describe and fit data to the circadian rhythms of chick melanocytes. These methods involve the fitting of exponentially decaying sinusoidal functions to the biochemical data recorded at intervals over days after various treatments. A major goal is to be able to isolate the effects of the treatments on the phase, frequency and amplitude of rhythms which continue after the cessation of light-dark cycles.

Analysis of Waves of Free Calcium in Glia. The Section has begun a collaboration with LCMN, NICHD, to investigate the nature of apparent free calcium waves in cultured astrocytes as detected by calcium-sensitive fluorescent dyes. The waves can be induced pharmacologically and are quite reproducible within a given cell. Attempts will be made to fit the data to passive diffusion models as well as to regenerative calcium release (*i.e.*, calcium-dependent calcium release). The Section's role will be in the areas of data analysis, model development, and experimental design.

ANNUAL REPORT

October 1, 1991 through September 30, 1992

Animal Health and Care Section, OD, DIR

National Institute of Neurological and Disorders and Stroke

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ANNUAL REPORT

October 1, 1991 through September 30, 1992

Animal Health and Care Section, OD, DIR

National Institute of Neurological Disorders and Stroke

Barton Weick, D.V.M., Ph.D., Acting Chief

During the past year, the Animal Health Care Section (AHCS) continued to work with and assist investigators of the National Institute of Neurological Disorders and Stroke (NINDS), Division of Intramural Research (DIR), to achieve the Institute's goals of better understanding, diagnosis, treatment and prevention of neurological disorders. Animal models and behavioral techniques were used for both spheres of biomedical investigation: clinical and basic research. Key areas of participation in animal model development by the Section's professional staff included:

1) Alzheimer's disease and related dementias; 2) continued research in inherited diseases, such as Huntington's, Batten's and Gaucher's; 3) epilepsy; 4) nervous system toxins; and 5) brain development and organization. The AHCS also serves as the Institute's liaison with organizations and institutions concerned with the ethical and humane care and use of animals in research.

AHCS has as its principle mission the establishment and maintenance of animal care and use program activities which support scientifically sound, animal-based research. Inherent in this goal is the recognition of the Institute's collective responsibility to conduct animal research in a legally, morally, and ethically acceptable manner with a high degree of sensitivity to humane concerns and animal well-being. Objectives which are essential to accomplishing this goal include: 1) developing program elements which meet or exceed the standards of the *NIH Guide for the Care and Use of Laboratory Animals* (NIH Guide) and assure full AAALAC (American Association for the Accreditation of Laboratory Animal Care) accreditation of the AHCS program consistent with the NIH Director's mandate; 2) implementing mechanisms for institutional oversight of animal research through the activities of the NINDS Animal Care and Use Committee (ACUC) which is constituted and functions according to PHS Policy on the Humane Care and Use of Laboratory Animals; 3) managing centralized animal care programs and facilities to provide a controlled, healthy, and appropriate environment for the maintenance of research animals and conduct of animal experimentation; and 4) providing professional veterinary expertise, guidance, and consultation in laboratory animal medicine and science to institutional executives, administrators, and scientists.

The AHCS manages the Institute's centralized animal holding and research facilities; assures an adequate supply of laboratory animals for Institute programs; consults with the Institute's scientific investigators to ascertain their animal care and husbandry needs and provides the services required; and provides technical assistance and guidance to Institute investigative staff on the biology and handling of laboratory animals.

The AHCS continues to operate as four service units: Small Animal Unit, Primate Unit, Research Support Unit, and Building 376 Unit. The Small Animal Unit maintains and operates four animal facilities; one each located in Buildings 9, 10, and 36 on the Bethesda Campus, and the Park 5 Building in Rockville, MD. Building 36 is a combined animal facility, providing housing and care to investigators from NINDS, NIMH, NHLBI, NICHD, and NIDCD under an intraagency agreement. The Primate Unit supports DIR investigators utilizing primates within Buildings 14 and 36 on the Bethesda Campus, the NIH Animal Center in Poolesville, MD, and in Building 49 when it opens in 1993. The Research Support Unit supports Institute investigators through the development of animal models of neurological diseases and by direct support of intramural research studies. The Building 376 Unit is located within the NINDS facility at Ft. Detrick, Frederick, MD. NINDS maintains one off-campus contract, the University of Southwestern Louisiana, New Iberia Research Center, for long-term housing and sampling of animals involved in AIDS research. In addition to this contract, NINDS has intramurally owned animals on loan to the Miami Metrozoo and the North Carolina Zoo.

Activities of the AHCS during Fiscal Year 1992 include:

- NINDS participated in the the NIH AAALAC Accreditation Plan. AAALAC has granted the NIH Intramural Program Provisional Accreditation for a period of twelve months. The NIH goal is to receive Full Accreditation in FY93. The only remaining major activity necessary to complete NINDS' accreditation preparation is the completion of facility renovations and repairs of all NINDS animal facilities.
- AHCS expanded its program of office computerization by implementing an electronic census system to assist in the management of animal orders and the NINDS animal study proposal database. This change significantly improved AHCS' capability to track and process animal orders for the NINDS ACUC and facilitated meeting the Animal Welfare Act and PHS Animal Welfare Policy requirements for increased accountability.
- Opened and implemented policies and procedures for the management of the newly renovated Building 14-D isolation cubicles for housing nonhuman primates in support of the animal research program of NINDS projects in the Veterinary Resources Program primate building.
- Established a sentinel program for monitoring the health status of Institute animals. By analyzing and interpreting the data gathered, the AHCS has been able to ascertain the general health status of research animals in each of its facilities and implement necessary preventive medicine practices to protect the health of Institute animals.
- Provided internal training sessions for AHCS technicians and caretakers to ensure the exchange of information on all phases of laboratory animal care. Two AHCS technicians were certified by American Association for Laboratory Animal Science (AALAS) at the Laboratory Animal Technician level and three technicians were certified as Laboratory Animal Technologists.

- Worked with investigators on animal disease detection, diagnosis, treatment and control. Discussed with numerous investigators the impact of subclinical infections of murine viruses on experimentation with immunocompromised rodents, particularly the nude mouse and severe combined immunodeficient (scid) mice.
- The newly created Research Support Unit has been involved in the development of animal models. Support has been in state-of-the-art therapeutic and diagnostic techniques, such as brain imaging, including positron emission tomography (PET) and magnetic resonance imaging (MRI) to pursue an understanding of the structure and function of the brain in health and disease.
- Interacted with and gave animal care and use advice to the 20 laboratories and research branches of the intramural program located at or near the NIH campus in Bethesda, MD and at Ft. Detrick, MD. This has included assisting investigators in the design of animal study proposals to ensure compatibility with sound scientific principles of animal experimentation and compliance with the PHS Policy, Animal Welfare Act, and other guidelines for the humane use of animals in research. This has also included the coordinating of training and assisting investigators in basic animal care and use techniques and animal model selection, as needed.
- AHCS staff participated in numerous PHS and NIH committees where NINDS representation was necessary. These committees included: NIH Animal Care and Use Committee, NIH Animal Program Advisory Committee, NIH Occupational Safety and Health Committee, Building 10A Animal Facility User's Committee and Building 10B Clinic Tower Animal Facility User's Committee. Extensive committee participation expanded AHCS' capability to provide input and assist in activities having an impact on NINDS animal research and related activities.
- Building 49 is scheduled for occupancy starting January, 1993. There are three main areas of planning and design being conducted with the assistance of the AHCS: primate housing that meets environmental enrichment requirements; small animal housing that meets requirements for disease detection and control; and surgical and radiological requirements for all species. These areas, as part of the coordinated occupancy program for Building 49, are of special interest to intramural investigators.
- AHCS recruited three students to assist with Section staff and Institute scientists on a variety of projects.

Proposed Course of Fiscal Year 1993:

In Fiscal Year 1993 the AHCS will explore the usefulness of visual and sensory evoked potentials in early diagnosis on animal models of human neurological diseases. Dr. Kay Jordan has joined the AHCS to continue her studies on the cortical atrophy of the primate brain of monkeys infected with simian immunodeficiency virus (SIV) and other aspects of virus replication, spread and pathology in the brain.

The AHCS will also be collaborating on a study of the human JC virus with Dr. Eugene Major, involving a recombinant virus that infects monkey glial cells.

Staff Presentations and Publications

AHCS staff continued during FY 1992 to participate in national and local professional and trade organizations in the fields of laboratory animal science and medicine through the publication of scientific papers and presentations in training courses and scientific meetings. Highlights of these activities are:

Rice, T, "What's My Line: Career Progression," Platform Session, National Capital Area Branch of the American Association of Laboratory Animal Science Annual Meeting, Ellicott City, Maryland, September 1992.

Perkins, S, Weick, BG, "Evaluation of the Use of Novel Objects by Adult Male Rhesus Macaques, Singly Housed in Horsfal Isolators," Annual Meeting of the American Association of Laboratory Science, October 1991.

Perkins, S, et al. Evaluation of the Use of Novel Objects by Adult Male Rhesus Macaques, Singly Housed in Horsfal Isolators, Laboratory Primate Newsletter, in press, 1992.

Collaborations Undertaken

Revision of a Sound Chamber to Induce Audiogenic Seizures in DBA/2 Mice .

Dr. Lowrey L. Rhodes, Jr. (AHCS)

Collaboration with Dr. Shun-ichi Yamaguchi (MNB). This collaboration is expected to finish in FY92.

Ratio of Kynurenic and Quinolinic Acid Accumulating in the Cerebrospinal Fluid after Intravenous Administration of Kynurenine.

Dr. Barton Weick and Dr. Alan Chedester (AHCS)

Collaboration with Dr. Vimala Sethy (ETB). This collaboration is expected to finish in FY92.

The Effects of Pentobarbital on Cerebral Damage Induced by Fractionated Whole Brain Radiation in the Primate.

Dr. Ruth Woodward and Ms. Lisa Berney, LATG (AHCS)

Collaboration with Dr. Aytac Akbasak (SNB). This collaboration is expected to extend into FY93.

Experimental Infection of Rhesus Monkeys with Human Herpes Virus Type 6 (HHV-6).

Dr. Lowrey L. Rhodes and Ms. Kristine Eckard, LAT (AHCS)

Collaboration with Dr. David M. Asher (LCNSS). This collaboration is expected to extend into FY94.

Regulation of Gene Expression in the Rat Hypothalamus II.

Dr. Venita Thornton (AHCS)

Collaboration with Dr. Scott Young and Dr. Susan Bachus (NIMH). This collaboration is expected to extend into FY94.

Alteration of Immunological Responses in Mice/Elimination of Staphylococcus Enterotoxin-Induced Response.

Dr. Venita Thornton, Mr. Dan Pare', LATG, Ms. Dawn Anuszkiewicz, LAT and Mr. Patrick Moran (AHCS)

Collaboration with Dr. Jon W. Marsh (NIMH). This collaboration is expected to extend into FY94.

The Mutant Niemann-Pick C Balb/C Mouse.

Mr. Dan Pare', LATG and Ms. Christine Lauter (AHCS)

Collaboration with Dr. Peter Pentchev (DMNB). This collaboration is expected to extend into FY94.

Study of Endothelial Regulation of Cerebral Vascular Smooth Muscle Tone in Vasospasm Induced by Subarachnoid Hemorrhage.

Dr. Ruth Woodward, Ms. Lisa Berney, LATG, Mr. Alphonse Cisar, LATG and Mr. Rob Lundgren, LATG (AHCS)

Collaboration with Dr. Gregory Thompson, Jr. (SNB). This collaboration is expected to extend into FY94.

Evaluation of Neurological Disease in SIV-Infected Monkeys with AIDS Using Magnetic Resonance Imaging.

Dr. Ruth Woodward, Ms. Lisa Berney, LATG, Mr. Alphie Cisar, LATG and Mr. Rob Lundgren, LATG (AHCS)

Collaboration with Dr. Kay Jordan (LCNSS). This collaboration is expected to extend into FY95.

Molecular Genetics of Neurointeractions.

Dr. Venita Thornton and Mr. Patrick Moran (AHCS)

Collaboration with Dr. Andres Buonanno (NICHD). This collaboration is expected to extend into FY95.

Toxicity Studies of TK-Producer Cells Injected into the Brain of Primates with and without Ganciclovir Administration.

Dr. Ruth Woodward, Ms. Lisa Berney, LATG, Mr. Alphie Cisar, LATG and Mr. Rob Lundgren, LATG (AHCS)

Collaboration with Dr. Zvi Ram (SNB). This collaboration is expected to extend into FY95

FY92 AHCS Projects

Maintenance of AHCS NINDS Primate Pool.

Dr. Barton G. Weick

Project is expected to continue into FY94.

Autogenous Blood Transfusions in Rhesus Macaques.

Dr. Ruth Woodward

Project is expected to continue into FY95.

ANNUAL REPORT

October 1, 1991 through September 30, 1992

NEURAL SYSTEMS SECTION

Basic Neurosciences Program, Division of Intramural
Research, National Institute of Neurological
Disorders and Stroke

Daniel L. Alkon, M.D., Chief

The Neural Systems Section takes a multidisciplinary approach to the question of how information is stored during associative learning and how it is made available for later recall. Biophysical and molecular mechanisms of associative learning are being analyzed in parallel for a mollusc (the sea snail *Hermisenda crassicornis*), the rabbit, and, most recently, the rat. Parallel analyses offer the opportunity for uncovering general cellular principles of learning and memory - principles which have been conserved over the course of evolution and which therefore could have relevance for human cognition. Parallel analyses also permit exploitation of critical experimental advantages unique to diverse species. For *Hermisenda* we have demonstrated a causal relationship of biophysical and molecular transformations within individual neurons to Pavlovian conditioning of a living animal. Causal relationships of cellular physiology and associative learning have not yet been approximated for any vertebrate preparation. Nevertheless, we have found evidence of biophysical transformations which are common to both mollusc and mammal. Furthermore, conservation of molecular memory mechanisms has also now been demonstrated with spatial maze learning in mammalian olfactory cortex.

Beginning with the snail and then continuing with mammalian brain structures, the laboratory has been attempting to reconstruct steps in memory acquisition and storage as they occur sequentially. One important step in the sequence involves persistent changes of K⁺ channels in neuronal membranes. An identified group of neurons, the CA1 cells (rather than individual identified neurons) was shown to have a distribution of conditioning-specific modification of K⁺ channels within hippocampal slices removed from rabbits on days after they had been conditioned. Virtually the same K⁺ channels change in snail neurons only after Pavlovian conditioning. Conditioning-specific changes of particular proteins have also been related to mRNA changes. The functional roles of these proteins as well as their identities are now being explored in the snail and in mammals. One protein, for example, has been linked to GTP-binding signal transduction. Another may have more importance for cell structure. These learning-induced changes of protein availability may represent an important step for consolidating, i.e., making the physiologic memory trace more permanent resulting in conditioning-specific structural changes of *Hermisenda* neurons. These latter changes, as studies with the formation of *Hermisenda* associations recently demonstrated, involve an apparent reorganization of the cell's terminal branches on which synaptic interactions occur.

Perhaps most important in all these efforts is the accumulating evidence that a remarkable similarity exists between molecular means of encoding learned associations in the snail, rabbit, and now the rat. Similar learning-specific regulation of K⁺ channels appears to occur at the molecular level for both mollusc and mammals. In the snail, these K⁺ channel changes result when calcium and diacylglycerol (DAG)-activated kinases catalyze phosphorylation of a low M.W. G-protein called cp20. This protein is a potent regulator of K⁺ channels as well as axonal transport. Kinase activation in the snail is mediated by synaptic convergence between the conditioned stimulus (CS) and unconditioned stimulus (UCS) pathways activated during Pavlovian conditioning. This synaptic convergence involves GABA-mediated inhibition that is transformed into excitation when stimulation of the CS and UCS pathways is precisely timed during training. Many of the critical molecular steps identified for snail associative learning have also been demonstrated in our laboratory for mammalian associative learning. These conserved steps include evaluation of Ca²⁺ and DAG, KC activation, and phosphorylation of the G-protein, cp20.

Such conservation of mechanisms and the synaptic networks in which they operate are suggesting common principles that can be described mathematically. Theoretical constructs based on these principles are now being incorporated into computer-based artificial networks which have already demonstrated remarkable pattern recognition capabilities.

Conservation of memory mechanisms at the molecular level may also provide the basis for clinical interventions and amelioration of pathologic symptoms. Such possibilities have become more likely in view of the recent discovery in our laboratory that Alzheimer's disease appears to involve defective voltage-dependent K⁺ channels similar to those we found involved in memory storage.

PROJECT DESCRIPTION

OBJECTIVES. The Section takes a multidisciplinary approach to the question of how information is stored during associative learning and how it is made available for later recall. The nervous system of *Hermisenda crassicornis* has proven to be a useful model for information processing at several levels: sensory transduction by photoreceptors and hair cells, analysis of synaptic circuitry, changes in synaptic circuitry produced by conditioning paradigms administered to intact animals, as well as to isolated nervous systems modified by conditioning, molecular transformations during long-term information storage and critical developmental stages for the neural networks of interest, as well as stages critical for learning. Techniques employed thus far to pursue these questions include simultaneous intracellular recording from multiple neural elements, paired stimulation of visual and vestibular pathways with stimuli similar to those in natural settings, iontophoresis of fluorescent dyes and electron dense materials, electron microscopy, voltage clamp analysis of macroscopic ionic currents, patch clamp studies of single channel ionic currents, and automated behavioral monitoring of intact *Hermisenda*. Other methods include mariculture, subcellular fractionation, protein phosphorylation analysis, uptake of neurotransmitter precursors, phosphoprotein characterization and purification, and immunologic protein identification. Patch clamp of membrane fragments of identified neurons is also being used to analyze enzymatic regulation of specific channels changed by learning to determine molecular mechanisms for encoding associatively learned information. Analogous protocols are also conducted with brain slices from neuronal aggregates which mediate classical conditioning of the rabbit, and most recently spatial maze learning of the rat. Such brain slices from both hippocampal and cerebellar regions have also been analyzed for subcellular distribution of enzymes as well as post- and pre-translational conditioning-specific modifications. Further related studies have been made of rat brain during spatial maze learning and odor recognition.

Recently, mathematical modeling has been used to quantitatively describe learning-induced modification of neuronal systems. Principles abstracted from biological memory systems are now being programmed into artificial network matrices with pattern recognition capability. Information processing at several levels is of interest:

- a. Sensory transduction by photoreceptors and hair cells
- b. Synaptic interactions between primary sensory receptors
- c. Synaptic interactions between primary and higher order neural elements
- d. Intersensory communication: e.g., synaptic interaction between the visual and gravitational sensory pathways
- e. Changes of synaptic interaction produced by conditioning, spatial maze learning and learned olfactory discrimination paradigms
- f. Membrane and synaptic properties modified by conditioning
- g. Identification of critical developmental stages of the neural networks studied, as well as stages critical for learning
- h. Biochemical mechanisms responsible for short- and long-term neural changes responsible for associative learning
- i. Structural changes during learning
- j. Integrative principles of neuronal array functions during memory acquisition, retention, and storage which may be incorporated into artificial networks

METHODS EMPLOYED

1. The nudibranch mollusc *Hermisenda crassicornis* is the principal "simple system" experimental preparation. The principal "complex system" preparation is the rabbit. Brain slices from critical areas within the rabbit brain have permitted analyses during the last few years of conditioning-induced biophysical and molecular changes parallel to those found in *Hermisenda*. Intracellular recording from several neural cells simultaneously, voltage clamp, and biochemical assays of phosphorylation, pre-translation function and cellular localization of enzymatic activity have been the main techniques used thus far. Patch clamp analysis of single ionic channels within identified neuronal membranes has also been conducted. Means for simultaneously stimulating the chemosensory, visual and vestibular pathways while recording intracellular potentials have been developed in our laboratory. Iontophoresis of fluorescent dyes (e.g., Procion Yellow) and electron dense materials (e.g., cobalt) are also being used extensively. Automated training and testing of animals has also been used to produce behavioral and correlated neuronal changes of learning.
2. Other methods allow additional biophysical, biochemical, electron microscopic, and developmental approaches to the problems of interest. These include voltage and patch clamp, mariculture, HPLC, protein sequence analyses, subcellular fractionation, two-dimensional microelectrophoresis, uptake of neurotransmitter precursors, etc.
3. Image analysis of autoradiographically labelled sections from vertebrate brain, histochemical techniques for identification of endogenous neurotransmitters, and *in situ* hybridization studies have all recently been introduced into the section's research program.
4. Capillary electrophoresis is now being used to examine the properties of cp20 and other proteins. The high mass sensitivity of capillary electrophoresis permits the analysis of cp20 from small regions of the *Hermisenda* CNS (approx. 5 cells) known to be involved in memory. Capillary electrophoresis will also permit developmental studies of this protein and studies of peptide hormones in *Hermisenda*. It may also be possible to adapt Capillary electrophoresis to perform microsequence analysis on proteins in the subpicomole range.
5. DNA technology is being used to clone the genes for cp20 and cp27, a structural protein from *Hermisenda* also affected by conditioning. We are also exploring DNA technology to create and examine transgenic *Hermisenda* to determine which proteins undergo an increase in synthesis after conditioning. To this end, we have constructed a rabbit cDNA library and *Hermisenda* genomic library.
6. Artificial membranes are being used to examine the complex interactions between protein kinase C and various signal-transduction lipids such as arachidonic acid.
7. Confocal microscopy, calcium-imaging, and molecular label imaging are used to evaluate ongoing structural and biochemical changes during associative learning.

MAJOR FINDINGS

Past work has focused on these major areas:

- a. Behavioral conditioning with neural correlates
- b. Cellular conditioning in isolated nervous systems
- c. Neural network analysis
- d. Persistent ionic channel modification and structural changes during associative learning and memory
- e. Molecular regulation of ionic channels, axonal transport, and neuronal structure during *Hermisenda*, rabbit, and most recently, rat learning and memory.
- f. Neural development
- g. Receptor physiology
- h. Artificial learning networks

BEHAVIOR

This program of the Section uses associative learning paradigms to produce persistent behavioral changes of the nudibranch mollusc *Hermisenda crassicornis* as well as of rabbits and rats. Quantitative assessments are made of the animals' responses to the conditioning paradigms. These assessments include precise dissection of generalized behavioral transformations into modification of individual muscular components of the behaviors. A full range of psychological manipulations have been used to clearly establish the sensitivity of the learning behavior to the exact temporal relationship of the training stimuli. With *Hermisenda* it has been possible to demonstrate associative learning with the same defining features observed for mammals. The learned behavior change is truly associative (i.e., random light and rotation do not produce the effect), persists for weeks after training and increases with practice. Stimulus specificity for this behavioral change was indicated by the fact that trained animals did not show changes in responsiveness to food or gravitational stimuli. Other features of vertebrate associative learning such as savings, a requirement for contingent stimuli, and extinction have also been demonstrated for *Hermisenda* associative learning.

One of the most demanding criteria for true Pavlovian conditioning (as shown for conditioning of the rabbit nictitating membrane) has been shown to be satisfied by *Hermisenda* conditioning. As a result of repeated pairings of the conditioned stimulus (CS) and the unconditioned stimulus (UCS) there is clear transference of the meaning (as defined behaviorally) of the UCS to the CS. The conditioned response (light-elicited foot contraction), which never occurs prior to the learning experience, approximates foot contraction stereotypically elicited by the UCS independent of training. It was further shown recently that there is an optimum interstimulus interval (ISI) between the CS and the subsequent UCS. The ISI curve generated closely approximates such curves characteristic of rabbit nictitating membrane learning and other vertebrate models of classical conditioning.

The eventual application of model preparation research to normal and dysfunctional human learning and memory is based on the assumption that animal and human learning and memory are related to each other. One of a number of questions that has yet to be addressed in model preparations is how long-term is long-term memory. To this end, experiments have been designed to test a rabbit's ability to recall and reacquire a conditioned response as long as 6 months after the original training experience. Experiments completed during the past year demonstrated

that the conditioning was intact 1 month after training, remained substantial 3 months after training, but could not be detected above baseline levels six months after training although relearning was rapid, i.e., there was clear evidence of "savings" of the conditioned response.

A second question that needs to be addressed is the apparent discrepancy in the complexity of human and animal associations. A series of experiments are being designed to involve multiple CSs being presented over a number of different intervals in order to test the limits of an animal's ability to form complex associations. Also during the past year we have initiated a human eyelid classical conditioning experiment that may permit imaging of human brain changes with positron emission tomography during learning.

Collaborative efforts involving more complex associations have already been undertaken using maze learning and olfactory conditioning in rats. The rat's ability to learn complex spatial tasks makes maze learning ideal for conducting experiments involving the substrates of complex learning and behavior. Olfactory conditioning is of considerable importance because it takes advantage of the complex stimulus combinations that can be created and the detailed knowledge that exists about the olfactory cortex.

NEUROPHYSIOLOGY AND NEUROPHYSIOLOGIC CORRELATES OF LEARNING

This program is concerned first with the identification of those neural systems relevant to learning capability. Multiple intracellular recordings from pre- and post-synaptic neurons have been employed within the visual, vestibular and chemosensory pathways of *Hermissenda* to establish a working knowledge of the critical neural systems and to describe how information flows in a stepwise fashion beginning with the sensory cells at the input, continuing through integrating cells, and finally to motor cells at the output. A similar approach is being taken with the rabbit hippocampus and cerebellar cortex, and critical afferent and efferent pathways within these structures. Neurophysiological correlates are then obtained (again for both the mollusc and mammals) for conditioned (as well as a variety of control) animals. These neurophysiologic correlates are recorded in intact animals, isolated nervous systems, and isolated neuronal membranes. Based on such correlates, electrophysiologic sequences are constructed to trace the transformation of the information (in electrical terms) by the neural systems.

Changes of conditioned stimulus-elicited neuronal activity have been recorded intracellularly from sensory receptors, interneurons and putative motoneurons in *Hermissenda*. That these correlated neuronal changes survive dissection of the animal after training indicated lack of vulnerability to generalized responses of the nervous system during its isolation. Observations from a number of different experimental procedures taken together, indicate that the neuronal changes in the visual pathway (mediating the response to the conditioned stimulus), correlated with learning, arise from a learning-induced bias in the relative excitability of photoreceptors within the *Hermissenda* eye. These observations further indicate that memory of the light-rotation association is actually stored by long-term modification of specific membrane channels (I_A and $I_{Ca2} + \gamma$) of the photoreceptors.

A sequence of biophysical and biochemical steps results from the integrated synaptic effect of the visual-statocyst network (in response to repeated light-rotation pairing) on these photoreceptors. The known synaptic interactions between photoreceptors,

interneurons, and motoneurons would be expected to transmit the measured increase of type B photoreceptor excitability by light so as to cause changes of motoneuron excitability (by light) in conditioned *Hermisenda*. Learning-induced increases of type B excitability, measured with blind procedures, in fact, were found to predict learning-induced changes of motoneuron excitability. That the excitability increases were intrinsic to the type B membrane (and not simply a passive reflection of other changes of cells presynaptic to the type B cells) was demonstrated by isolating the type B somata from all other neurons. Type B somata, with all synaptic activity and impulse-generating membrane eliminated by axotomy showed enhanced responses to light and current injections when taken from conditioned but not control animals during the learning-retention period. These learning-induced changes of excitability which were intrinsic to the type B somata were of sufficient magnitude to account for increases of impulse activity during and after a light step presented to intact nervous systems of conditioned but not control animals. The increases of type B impulse activity were also of sufficient magnitude to substantially account for the measured changes of motoneuron responses to light in conditioned animals. We also have recently found that the medial type A photoreceptor shows an intrinsic decrease of excitability (in contrast to the type B cell's increased excitability) after training.

Current injected into the hair cells to simulate the effects of natural sensory stimuli could be paired with light to mimic the stimulus regimen of Pavlovian conditioning. Such simulation in isolated nervous systems or living animals produced the same changes of type B excitability that occurred during Pavlovian conditioning of the living animals with natural stimuli. In the living animals, light stimuli paired with intracellular current injections produced long-lasting behavioral changes days after cell impalement and injection. Control injections and sham operative procedures produced no changes of type B excitability or behavior.

To explore possible analogies between vertebrate and invertebrate mechanisms of learning, hippocampal slices were isolated from brains of rabbits which had learned during classical conditioning of its nictitating membrane. Membrane properties of identified cell types within the slice were assessed for conditioned as well as control animals. Because these cells share many ionic currents with the type B cells (which store associative learning in *Hermisenda*) they were thought to possibly be useful for testing parallels of learning mechanisms in different model systems.

On days after conditioning (or control regimens) electrophysiologic properties of CA1 neurons within the slices were assessed. I_C of conditioned cells compared to pseudoconditioned and naive cells were shown to be reduced in the rabbit as was previously shown for I_C (as well as an early current, I_A) in *Hermisenda*. This demonstration not only shows a remarkable conservation but also that such a mechanism is intrinsic to the CA1 cells within isolated slices (and thus cannot be viewed as a passive reflection of memory storage elsewhere in the rabbit brain). Furthermore, only those rabbits which actually acquired the classically conditioned response had CA1 pyramidal cells (studied in slices) with reduced I_C . These data link the I_C reduction observed *in vitro* to conditioned response acquisition observed behaviorally. Another electrophysiologic correlate of rabbit classical conditioning has also been obtained for the CA1 cells: conditioning-specific enhancement of the summation of synaptic potentials. This synaptic difference, not apparent for single postsynaptic potentials (PSPs), is characterized by increased depolarization during 50Hz, 300 msec stimulation of the Schaeffer collaterals in cells from conditioned relative to control animals. This enhanced summation correlates with I_C reduction which probably occurs on post-synaptic CA1 dendrites.

Recently, analysis of memory-specific differences in mammalian brain slices has been extended to the rabbit cerebellum. Intracellular recordings made in rabbit Purkinje cell dendrites of lobule HVI revealed membrane potential, action potential, and input resistance values that were similar to values described for vermal Purkinje cells in guinea pig and rat. Activation of parallel fibers and climbing fibers produced synaptic potentials also similar to those previously described in other species. A comparison of Purkinje cell dendritic activity in lobule HVI for animals subjected to classical conditioning, explicitly unpaired, or no treatment procedures, revealed a significant decrease in the threshold for dendritic spikes in cells from conditioned animals versus cells from unpaired and untreated animals. There were no significant differences in membrane potential or input resistance among the treatment groups. The present results suggest a conditioning-specific increase in Purkinje cell dendrite excitability in lobule HVI which may have consequences for classical conditioning of the rabbit nictitating membrane response.

In vitro stimulation and recordings from Purkinje cell dendrites using current injections and GABA application have been used during the past year to analyze the electrophysiologic consequences of precisely timed climbing and parallel fiber inputs. We have also obtained evidence during the past year that GABA_A receptors are located predominantly on the Purkinje cell somata, and that GABA_B receptors are located predominantly on the dendrites. Other experiments suggest the long-term modifiability of these GABA_B receptors.

A major new finding of our laboratory involves a total transformation of the GABA synapse during associative learning. Pairing of a light stimulus which depolarizes the postsynaptic type B cell with a hair cell stimulus (e.g., rotation) which releases GABA from the hair cell presynaptic endings changes the normal synaptic inhibition into synaptic excitation. This long-term transformation of GABAergic inhibition into excitation persists for hours after induction. It provides a critical missing link between short- and long-term memory since K⁺ current reduction and increased excitability of the type B cell can now be understood as a direct consequence of the GABA synapse transformation.

BIOCHEMISTRY

Our research on the biochemistry of learning and memory has concentrated on several interrelated areas: (1) the role of signal transducing G proteins, such as cp20, and calcium-binding proteins in memory storage; (2) signal transduction lipids such as arachidonic acid, and other membrane lipids; (3) oncogene products, in particular ras and c-fos; (4) protein kinase C; (5) DNA transcription and its regulation as a basis for memory storage; and (6) the potassium channel and its regulation. A complex chain of biochemical events involving these six pathways appears to be involved in memory storage in both *Hermisenda* and rabbit. For example, the interaction of Ca²⁺ and signal-transduction lipids with protein kinase C, perhaps acting via oncogene products, can result in effects on the cell nucleus, which may be involved in memory storage. The G protein cp20 affects the potassium channel directly, but also may affect long term storage by affecting protein translation. Clarifying the manner in which these events interact to store information in the neuron will be a major focus of our research.

Previous experiments conducted on *Hermisenda* in our laboratory demonstrated that associative conditioning increases the amount of ³²P-labeling in a single 20 kDa protein. These experiments involved isolating nervous systems after conditioning, incubating them in artificial sea water (ASW) in the presence of ³²P inorganic

phosphate (^{32}Pi), then dissecting the attached eye, and analyzing the proteins on SDS gel. A number of experiments clearly demonstrated that changes in protein phosphorylation of specific proteins, including cp20, could occur after conditioning, cell depolarization, and pharmacological treatments. For example, phosphorylation of a protein of 20kDa and pI of 4.4, along with phosphoproteins of Mr 25 and 56 kDa, was increased by incubation of isolated total nervous systems in high-potassium (100 or 300 mM K^+) ASW, a condition which mimics the prolonged depolarization of specific cells which occurs during conditioning. The 25 kDa protein was also affected by 4-aminopyridine treatment. Phosphorylation of the 56 kDa protein was increased two-fold by high K^+ , possibly due to the action of a phosphatase. Two of these three proteins (20 kDa and 56 kDa) were also phosphorylated in response to exogenous phorbol esters, which activate protein kinase C. Phorbol ester treatment also resulted in the phosphorylation of a 21.5 kDa and a 62 kDa phosphoprotein. These proteins were also present in eyes isolated from ^{32}P -labeled CNS.

A relationship to learning of phosphorylation of specific proteins (in response to activation of Ca^{2+} dependent kinases) has been supported by a large number of experiments. These include the effects on the same K^+ channels modified during learning by a variety of manipulations affecting Ca^{2+} -dependent phosphorylation such as injection of Ca^{2+} -calmodulin-dependent Type II kinase, protein kinase C (PKC), inositol trisphosphate, elevation of intracellular Ca^{2+} , application of a diacylglycerol (DAG) analog, and perfusion with arachidonic acid (AA). All these experiments taken together provide strong evidence that associative learning (as well as a cellular model of learning called long-term potentiation [LTP]) begins with elevation of Ca_i^{2+} , DAG, and AA, resulting in the synergistic activation of Ca^{2+} -calmodulin-dependent Type II kinase and PKC.

The possible involvement of proteolytic activation of protein kinase C was suggested by voltage-clamp experiments. Injection of purified protein kinase C increased the K^+ currents, whereas injection of PKC after a prior injection of leupeptin, an inhibitor of Ca^{2+} -dependent proteases, had the opposite effect, i.e., a reduction of K^+ currents. These experiments indicated that protection of PKC from proteolysis, either by membrane insertion or addition of protease inhibitors, is necessary for PKC to effect a blockade of the K^+ channels, and also suggested that proteolyzed PKC if constitutively present, could normally act to maintain the cell in a hyperpolarized state.

Other experiments implicating PKC activation in memory storage involved measurements of PKC activity in mammalian brain structures after training and control paradigms. For example, membrane PKC activity increased in rabbit CA1 hippocampus after tone-eyeblink conditioning. Although total levels of PKC were unchanged, the percentage of PKC in the membrane, measured after partial purification on L-threonine phorbol ester, increased from 42 to 63%. Imaging studies of phorbol ester binding in rabbit hippocampus, rat hippocampus, and identified *Hermisenda* neurons have confirmed the generality of this phenomenon.

Recently, we have found that arachidonic acid and diacylglycerol exert a potent synergism to activate PKC in model membrane bilayers. We have also demonstrated that arachidonic acid, in combination with diacylglycerol, can also mimic associative memory in a cellular *Hermisenda* model of associative conditioning. Similarly, perfusion of rat hippocampal slices with diacylglycerol followed by arachidonic acid and EPSPs evoked by Schaeffer collaterals stimulation, induced a marked increase in EPSP amplitude. These experiments demonstrate that arachidonic acid-

diacylglycerol synergism may play a role in neuronal changes during learning in a variety of systems.

Other studies have recently involved synaptosomes isolated from the CA1 hippocampal region of conditioned rabbits. There were no intrinsic differences in membrane-bound PKC levels, compared with naive rabbits. However, treatment of synaptosomes from conditioned rabbits with phorbol ester PDBU and depolarization with high K⁺ results in markedly higher levels of PKC bound to the membrane. In synaptosomes from untrained rabbits, PDBU and high K⁺ have no effect. This suggests a difference in PKC between naive and conditioned rabbits that is reflected in increased ability of the cytosolic PKC to be translocated to the membrane.

During the last year, efforts have been made to analyze biochemical steps leading to PKC activation and release of intracellular Ca²⁺. To this end, an assay method using fluorescent substrates has been established to monitor phospholipase activity within slices of mammalian hippocampus and cerebral cortex.

To investigate whether any specific proteins were changed following associative conditioning, we trained *Herrissenda*, dissected the eyes, and quantitated the proteins on AX-300 HPLC. In some cases the eyes were also incubated with ³H amino acids and/or ³²Pi. Four proteins were consistently changed in the conditioned group. The peak area of one of the proteins, designated conditioning-associated protein 27 or cp27, was found to be decreased by about 60% in conditioned eyes, while a second protein, cp20, was increased about 3-fold. This increase was due to an increase in its phosphorylation state, since the rate of ³H-amino acid incorporation was not increased but actually decreased by 30%. The phosphorylation of two other proteins, cp26 and cp18, were also increased approximately 2-fold following conditioning. Analysis of the cp20 on size-exclusion HPLC, SDS gel electrophoresis, and reversed-phase HPLC showed that the cp20 peak consists of a single 20 kDa protein, and that cp20 from paired eyes was the same as cp20 from naive eyes and CNS. This eliminates the possibility that the increased peak size was due to a new protein that co-chromatographed with an old peak, and supports the idea that the increased peak size was the results of a change in a preexisting protein.

We now have several pieces of evidence indicating that cp20 is a low-molecular weight G protein (1) assaying each fraction from the ion-exchange HPLC column for GTPase and GTP-binding activity, shows a sharp peak of activity that coincides with cp20; (2) rechromatography of this peak on size-exclusion HPLC shows a sharp peak of GTPase and GTP-binding activity at the 20 kDa region. The stoichiometry of GTP binding was 0.95 mol GTP/mol protein; (3) purified cp20 reacts with ³²P-labeled azidoanilido-GTP, a photoaffinity label for G proteins, to form a covalently-labeled derivative; (4) probing a nitrocellulose blot of purified cp20 analyzed by SDS gel electrophoresis with ³²P-GTP showed a single ³²P band on autoradiography, at 20 kDa, coinciding with a single protein band; (5) cp20 is retained on GTP-agarose, an affinity column material selective for GTP-binding proteins.

Injecting purified cp20 into the photoreceptor neurons from which it was isolated causes a dramatic, immediate increase in the response to light. This effect is similar to that observed after conditioning. No other biomolecules, including ras, various protein kinases, or proteases, can produce this effect at the low concentrations at

which cp20 affects these cells. The biophysical basis for this excitatory effect was found to be a blockage of the same two K^+ channels which are partially blocked after associative conditioning, i.e., I_A and $I_K + -Ca^{2+}$. Voltage-clamp analysis of these cells indicated that cp20 reduced I_A and $I_K + -Ca^{2+}$ by 50% and 70% respectively. The inhibition of the currents was maximal five minutes after injection, and remained constant for at least 15 minutes.

The effects of ras are similar to those of cp20, although ras is much less potent than cp20. Ionophoretic injections of Ha-Ras into *Hermisenda* LP1 cells, for example, progressively reduced the amplitudes of the potassium currents I_A by 30-40% and $I_K + -Ca^{2+}$ by 40-60% after a delay of 20 min, possibly representing the time required for post-translational modification or membrane association of the ras. Virally transformed ras-va 112 (v-ras) was much more effective than the nontransforming form (c-ras) at reducing the currents, which showed no sign of returning to normal after 60 min. A similar difference between c-ras and v-ras was observed on the Ca^{2+} currents, with the effects of v-ras being at least twice as large as c-ras, and showing no signs of reversal at 60 min. Similarly, the effects of cp20 on K^+ currents more closely resembled those of the virally transformed ras than normal c-ras in their intensity as well as the time duration.

We have also found that cp20 exists in a variety of species besides *Hermisenda*, including squid, cuttlefish, octopus, and rabbit. Tone-eyeblink conditioning of rabbit caused a decrease in the labelling of three G-proteins, including a 20 kDa G-protein, in CA1 hippocampus by about 30-40% as well as a decrease in labeling of a 38 kDa G protein. Thus, changes in G proteins similar to those seen in *Hermisenda* appear to occur in mammalian brain as well.

Recent data suggest that the *Hermisenda* G protein cp20 can also affect axonal transport. Application of $1\mu M$ cp20 to isolated crab walking leg neurons reduced the number of particles moving in the retrograde direction, as observed by contrast-enhanced video microscopy, by approximately 45%. A role in active transport could explain the axonal morphologic changes observed after conditioning in *Hermisenda* photoreceptor cells. Partial sequence information obtained from squid cp20 also indicates similarities between cp20 and calcium-binding proteins, as well as between cp20 and other low-molecular weight G proteins. Currently, experiments are underway to sequence cp20 and to generate polyclonal antibodies to cp20 and cp27.

Conditioning also increases the mRNA content of the *Hermisenda* eyes; 24 hours after 3 days of training, the poly(A)⁺ mRNA in the eye, which consists of 5 neurons, a few small epithelial cells, and a lens, was increased 2-fold. The mRNA levels returned to normal by 4 days after the end of conditioning. Analysis of agarose gel patterns indicated that at least 21 species of mRNA were significantly increased. Large increases were also observed in unprocessed high molecular weight RNA. In preliminary experiments, we have found that purified cp20, when added at $1\mu M$ to a rat hippocampal protein synthesis system, increases the rate of ^{32}P incorporation into mRNA by 4-fold. Thus, the earlier increases in mRNA in conditioned *Hermisenda* could be a direct result of the increased cp20 in those cells. During the past year, efforts to sequence and further characterize cp20 have included its identification in squid optic lobe. We have found that cp20 of squid or *Hermisenda* can be isolated predominantly as a 40 kD dimer unless purified with DTT. The dimer form has a lower thermal stability than the monomer form. Its inactivation curve is biphasic, with a brief activation phase followed by a slower inactivation phase. The two forms also differ in their enzyme kinetic rates. The stoichiometry of GTP binding

to the monomer was 0.92 mol GTP/mol. The protein has a weak cross-reactivity with an antibody to the 35 kD G γ subunit. We are currently using DNA probes against this subunit to attempt to clone cp20.

IMAGING

To display learning-specific changes across small and large ensembles of neurons, our strategy has been to exploit the critical role of enzymes such as protein kinase C in the molecular chain of events that lead to associative learning. Radioactively labeled phorbol ester, for example, has served as a probe for membrane-associated PKC in animals that have undergone various behavioral paradigms designed to assess learning. More recently, we have begun to use fluorescent probes for PKC and phospholipase A (PLA) to study the analogous molecular events in brain slices, dissociated hippocampal pyramidal cells, and *Hermissenda* eyes, using state-of-the-art confocal microscopy.

In our initial studies, we were able to demonstrate a large and reproducible increase in the amount of membrane-associated PKC 24 hr after classical conditioning procedures in rabbit. Radioactive phorbol ester which binds to membrane-associated PKC (3H-phorbol-12, 13-dibutyrate; PDBU) revealed steady-state shifts of PKC distribution (within the CA1 cell field of the mammalian hippocampus) during retention of a classically conditioned behavioral response. In subsequent studies, autoradiographic images of 3H-PDBU were analyzed for changes in the label along orthogonal transept lines within the CA1 cell field. Images were compared between groups of animals and demonstrated a significant movement of label into the region of the basilar dendrites some 72 hr after the learning experience.

The functional role of the hippocampus was also examined using the quantitative autoradiographic technique by testing rats in a spatial and cued discrimination task. The data obtained demonstrated that the hippocampus was involved in the acquisition of both the cued and spatial discriminations. As with the rabbits, the phorbol labeling techniques were able to pick out a learning-specific change of PKC distribution within the hippocampus. More recent experiments using the same methodology have demonstrated learning-specific changes in the distribution of PKC in rabbit hippocampus during the initial acquisition phase of Pavlovian conditioning and in the B cell of *Hermissenda*, 24 to 48 hr after Pavlovian conditioning. During the past year, we have demonstrated (in collaboration with Dr. David Olton at Johns Hopkins University) a clear dissociation of hippocampal PKC distributional changes depending upon whether the learned task was hippocampal dependent and/or required the use of spatial cues for its solution. Additionally, we were able to show, in collaboration with Jim Bower's laboratory at Cal Tech, for the first time the involvement of the olfactory cortex in an olfactory cue-dependent task (and not an auditory cue-dependent task) using the [3H]-phorbol ester autoradiographic method. Finally, other very recent studies are uncovering new molecular labels that should be able to follow the function of neuronal assemblies with unusually good temporal resolution.

ANATOMY

The cellular anatomy aspect of the Section's programs contributes in several ways to the various levels of inquiry into the learning process already mentioned. Ultrastructural measurements of the cells and their synaptic interconnections have provided further definition of the relevant neural systems. Activity-dependent uptake of radioactive labels within these systems has been monitored

autoradiographically. Morphometric techniques, together with serial sections and computerized reconstruction, have been employed to uncover structural manifestations of the biophysical and biochemical changes already shown for neurons within conditioned but not controls animals.

Recently, our laboratory demonstrated physical changes within the branching structure of the *Hermisenda* B cell as a result of learning. A single identified neuron, the medial B cell, was filled with Ni^{2+} -lysine to define its complete structure. Several days after conditioning (but not control procedures) the terminal branches of the type B cell were found to occupy a reduced volume of three dimensional space within the *Hermisenda* nervous system. This contraction of the terminal branch volume was clearly related to a reduction of K^{+} currents as well as behavioral performance.

During the past year, injections of cp20 into the *Hermisenda* type B cell were shown to produce structural changes of terminal branches similar to those found after classical conditioning. Both cp20 injection and classical conditioning produce contraction of "focusing" of the type B terminal branches on which synaptic interactions are localized.

BIOPHYSICS

Extensive voltage-clamp studies of the soma membrane of the isolated *Hermisenda* type B photoreceptors have now been conducted. These cells (of which there are three in each eye) were shown to undergo primary biophysical changes during associative learning: i.e., changes intrinsic to the soma membrane were observed. Because these cells, via synaptic interactions, affect most, if not all, neurons within the visual pathway, their changes can be responsible for the animals' associative learning behavior. In addition to two light-induced currents ($I_{\text{Na}^{+}}$, $I_{\text{Ca}^{2+}-\text{K}^{+}}$), these voltage-clamp studies demonstrated two voltage-dependent outward K^{+} currents: a large, fast, early current and a slow, late current. The large, early outward K^{+} current, I_A , and $I_{\text{Ca}^{2+}-\text{K}^{+}}$ were found to be greatly reduced in associatively trained but not control animals. This decrease of specific dark K^{+} currents with learning is consistent with a number of previous observations. It explains, for instance, the increased input resistance of type B cells (after the somata were isolated from their axons and synaptic endings) from trained animals. A decreased I_A and $I_{\text{Ca}^{2+}-\text{K}^{+}}$ specific to conditioned animals can also account for an enhanced type B voltage response (during and following light steps) which in turn, via known synaptic interactions, can account for the learned behavior. Conditioning was shown to also significantly reduce $I_{\text{Ca}^{2+}}$, a voltage-dependent inward Ca^{2+} current, measured across the type B soma membrane 1-2 days after prolonged training with paired stimuli (but not control paradigms). Recent voltage-clamp analysis of CA1 pyramidal cell ionic currents have revealed remarkable similarities of learning induced changes between mammalian and the *Hermisenda* preparations. These and other results from our studies of Purkinje cell dendrites in the cerebellum provide strong evidence of conservation of memory storage mechanisms involving ionic flux through membrane channels.

Other experiments have been directed at establishing a sequence of biophysical steps which lead to the observed long-term membrane changes of the type B cell and mammalian neurons. Direct measurement of calcium levels within the type B cell suggested that elevation of cytoplasmic Ca^{2+} during the long-lasting depolarization (LLD) is voltage-dependent, and thus should also be enhanced when light is paired with rotation during the conditioning procedure. Taken together, the

data of a variety of other experimental analyses indicated that the following sequence of biophysical steps occurs during acquisition and retention of the associative learning: (1) pairing of light and rotation cause synaptic depolarization which enhances in a nonlinear manner the voltage-dependent LLD which arises in part from a voltage-dependent Ca^{2+} current; (2) repetitive pairing results in cumulative membrane depolarization and elevated intracellular Ca^{2+} ; (3) elevated intracellular Ca^{2+} together with other intracellular second messengers causes long-lasting inactivation of I_A and I_C ; (4) reduced I_A and I_C caused increased input resistance and enhanced LLD responses of type B cells.

In addition to the commonly employed intracellular recordings and two-electrode voltage clamp techniques, the patch-clamp methodology has been successfully adapted to extend the study of ion channel regulation in *Hermisenda* nerve cells. Patch-clamp experiments are performed using an isolated eye preparation where two or more photoreceptors can be identified. The photoreceptor preparation is obtained by a combination of enzymatic treatment (protease and dispase) and microdissection. The light responses observed in this preparation are virtually indistinguishable from the responses obtained with intracellular electrodes in the traditionally employed axotomized preparation (which is subjected to gentler enzymatic and dissection procedures).

At the single channel level, we have in the past studied the basic features of two distinct K^{+} channels present in the soma membrane of the B photoreceptor. In the cell-attached configuration, these channels have conductances of 42 pS and 64 pS, respectively. These channels also differ in their kinetic properties. The percentage of open time at resting potential (i.e., pipette potential = 0 mV) is around 35% for the 64 pS channel and around 12% for the 42 pS channel. Recently, we have studied the possible regulatory mechanisms of these K^{+} channels, with special attention to the role of PKC and classical conditioning. We have found that PKC activation dramatically and selectively modified the behavior of the 64 pS channel, while the 42 pS channel remained unaffected. The use of the PKC inhibitor H-7 resulted in a 65% reduction of the phorbol effect. Inside-patches obtained from phorbol preincubated cells likewise showed this effect, but the effect was not observed when phorbol was directly added to cell-free patches obtained from nontreated cells. These results suggest an important and long-term effect of PKC on the 64 pS channel. They also indicate that cellular integrity is required for PKC activation/translocation by phorbol ester. It was further established that only one of the K^{+} channels (the 64 pS) was selectively modified, and in a similar manner by both PKC and classical conditioning.

Channel activity recordings were also obtained from naive, unpaired and conditioned animals. The 64 pS channel appeared with significantly lower frequency (15.4%) in patches performed on cells from the conditioned group, compared to the controls (naive: 59% and unpaired: 55.6%). In addition, when present, the 64 pS channel showed a lower % open time and an increased interval between opening bursts, in cells from conditioned animals. The 42 pS channel was observed with about the same frequency in all three groups. Thus we have shown for the first time at the single channel level regulation of K^{+} channels by classical conditioning and the important role that PKC activation may play in this process. Currently, additional experimental approaches are used to study with further details the above mentioned K^{+} channels, as well as the identification of the unitary channels responsible for the previously described macroscopic K^{+} conductances. Polymerase chain reaction (PCR) technology is currently being employed to clone K^{+} channels of

Hermisenda to further characterize their role and regulation during memory storage.

Integration of biochemical biophysical techniques has allowed us to study the calcium-dependent enzymatic phosphorylation of ion channels that leads to basic mechanisms for memory storage in *Hermisenda* and mammals. Second messenger systems have been studied at the single channel or macroscopic current level. These systems include molecules such as PKC, C-kinase, fatty acids, small G proteins and calcium.

With current-clamp and voltage-clamp recordings of the postsynaptic B cell in *Hermisenda* we have also recently characterized an entirely new GABAergic synaptic input from the hair cells, that can regulate newly described ion conductances on the B cell. Activation of this synapse by hair cell stimulation or topical application of exogenous GABA hyperpolarizes (i.e., inhibits) the postsynaptic type B cell by (1) opening GABA_A-mediated Cl⁻ channels, and (2) opening GABA_B-mediated K⁺ channels. After the postsynaptic B cell is depolarized by light or current injection together with GABA, subsequent GABA applications or presynaptic hair cell impulses cause depolarization of the B cell. During the last year extensive voltage clamp analyses uncovered the ionic basis of this remarkable synaptic transformation from inhibition to excitation. After light-GABA pairings (3 are sufficient) GABA no longer opens Cl⁻ or K⁺ channels, but instead closes K⁺ channels thereby causing depolarization. Fura-imaging experiments have demonstrated during the last year that GABA closes K⁺ channels by releasing Ca²⁺ from intracellular stores. Additional experiments during the last year have shown that conditioning the snail enhances and prolongs this Ca²⁺ signalling even several days after training. This new "memory trace" has important implications for learning by networks, both biological and computer-based.

Computer-aided modeling of the ion currents recorded in the isolated cell body of the B cell had also yielded a comprehensive account of their impact on the overall cell excitability, and the model has also been capable of reproducing the associative learning-induced changes in excitability, by modulating the same currents.

ALZHEIMER'S DISEASE

Because Alzheimer's disease has, for many years, been considered to characteristically cause memory dysfunction, our laboratory has begun to assess dysfunction of K⁺ channels in Alzheimer's patients. Fibroblasts obtained from Alzheimer's (AD) patients, aged-matched controls, and young controls were analyzed with two separate technologies particularly to assess K⁺ channel function. These analyses have uncovered striking differences between Alzheimer's and control K⁺ channel function. The Alzheimer's fibroblasts show a complete absence of a 113 pS channel which was recorded with patch-clamp techniques from almost all control cells. A 162 pS K⁺ channel, absent from old cells and sometimes present in the young cells, is consistently present in the Alzheimer's cells. The 113 pS K⁺ channel absent from the Alzheimer's cells was sensitive to TEA. Calcium signals imaged within the fibroblasts in response to TEA were, like the 113 pS channel, entirely absent from the Alzheimer's but not the control cells. Other calcium signals in the Alzheimer's fibroblasts, however, were not different from those of the controls. Thus, both the patch-clamp and calcium-imaging results implicated K⁺ channel dysfunction in Alzheimer's disease.

ARTIFICIAL COMPUTER-BASED LEARNING NETWORKS

In collaboration with the contractor known as ERIM (Environmental Research Institute of Michigan) we have been developing artificial computer-based networks modeled after the biological learning networks we have analyzed in molluscan and mammalian species. Dystal (DYNAMICALLY STable Associative Learning) is one such artificial neural network based on the features of learning and memory identified in *Hermisenda* and rabbit hippocampus. Dystal's local learning rule, the concept of patches and other design principles were derived from biological mechanisms. As a consequence, Dystal has a number of desirable mathematical properties: a theoretical storage capacity of b^n non-orthogonal memories, where b is the number of discrete values and n the number of output neurons: a computational complexity of $O(N)$: monotonic convergence: and the ability to learn, store, and recall associations among noisy, arbitrary patterns.

Dystal classifies by learning associations between patterns in a training set and their desired classification. Because the network is a model of associative learning, we call the pattern to be classified the CS (conditioned stimulus) and the desired classification of the UCS (unconditioned stimulus). Structurally, Dystal has two separate input pathways: a CS input pathway for the CS pattern and the UCS input pathway for the UCS to be associated with the CS pattern; CS pattern; both the CS and UCS input elements are connected to the output layer via a series of patches. The entire set of patches collectively stores all of the patterns that Dystal learns; however, each patch stores only a single association between a part of a CS input pattern and one component of the UCS. Learning (patch creation and modification) occurs when presentation of CS pattern is followed by the presentation of a UCS according to non-interactive learning rules. During testing, no UCS is presented, thus no learning occurs; otherwise, training and testing are the same.

The excellent performance resulting from a biologically motivated design is demonstrated using hand-written ZIP code digits and hand-printed Japanese Kanji characters. After a single pass through the training set of segmented digits, Dystal correctly classifies 98% of previously unseen hand-written digits. The performance is robust to changes in global parameters. When similarly trained to classify Kanji characters, it is able to learn 40 people's handprinting of 160 different characters to 99.8% accuracy, a task analogous to learning the latin characters in 40 different fonts. When tested on handprinting of 120 people different from those who wrote the training set, Dystal correctly recognizes 90% of the characters.

PROPOSED COURSE OF PROJECT

1) Precise analysis of *Hermisenda* synaptic interactions between cells within the aforementioned neural networks will be continued with the techniques of intracellular recording and iontophoresis. Particular emphasis will be placed on electron microscopic visualization and reconstruction of cell contacts aided by distribution of hydrogen peroxidase within axons and terminal branches. These studies will not be limited, however, to the networks already discussed. Additional motor units within the sensory pathways (visual, statocyst, and chemosensory) will be identified.

2) Anatomic, as well as additional electrophysiologic, correlates of behavioral and developmental changes will be sought in *Hermisenda*, the rabbit, and the rat. Morphometric techniques together with serial sectioning and computerized reconstruction should continue to uncover structural manifestations of the

biophysical and biochemical changes already shown for neurons within conditioned but not control animals. Using voltage-clamp techniques, cellular mechanisms responsible for the learning model will be further analyzed. Particular attention will be given to a study of the potential-dependent currents believed to underlie, at least in part, the observed behavioral changes. Also of special interest will be the morphologic, biophysical, and biochemical changes responsible for storing associatively learned information for many weeks. The relationship of these relatively permanent changes to developmental neuronal changes will also be investigated.

3) Biochemical and pharmacologic analyses of relevant neural systems will continue. We will continue to study subcellular and/or biochemical loci at which primary behaviorally meaningful changes occur. The type B photoreceptor, hippocampus, and the lobule HVI area of the cerebellar cortex will provide the main foci for this work.

We plan to identify the mechanisms leading to the observed changes in protein phosphorylation specific to learning in *Hermisenda*, the rabbit, and the rat, i.e., involving changes of adenylate cyclase, phosphodiesterase, protein kinase and/or phosphatase activities. Other mechanisms of posttranslational modification will also be explored. We also will study the synthesis and modification of gene products in these nervous systems by means of molecular biologic techniques. Studies will also be continued to isolate the proteins which undergo transformation during learning. The relationship of such proteins to the structure of membrane channels as well as their regulation will continue to be investigated.

4) Analytic tools (via the use of modern molecular biology) such as monoclonal antibodies will be used to scan large neuronal arrays in mammalian brain slices. "Images" of such arrays in these slices will then be reconstructed to yield three-dimensional representations of conditioning-specific shifts of neuronal system metabolism. Such "images" represent an extension of methodology already proven to be highly effective (with, for example, radioactive phorbol ester) for assaying memory-specific functions of large neuronal arrays.

5) Mathematical models of *Hermisenda* classical conditioning will be generated to provide quantitative description of membrane ionic currents, neural systems and behavior. Mathematical transformations will be derived to allow interfacing of quantitative descriptions at these different levels of biological complexity. Artificial systems will be designed based on *Hermisenda* models (as well as confirmed generalities to vertebrate brain slices). Successful creation of such artificial systems will not only have inherent practical value, but will also serve to indicate how complex biological systems (not amenable to comprehensive description) may function.

6) Differential absorption spectrophotometry will be further utilized for localizing intracellular fluctuations of cytosolic Ca^{2+} as they occur during different phases of the learning process. Cytochemical measurements within individual neurons will be further applied to reveal neurochemical means of amplifying the Ca^{2+} -dependent modulation of the channels during associative learning.

7) Behavioral experiments will be continued to further determine the comparability of the *Hermisenda* associative learning model to associative learning defined for more evolved species.

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3) Biochemical and pharmacologic analyses of relevant neural systems will continue. We will continue to study subcellular and/or biochemical loci at which primary behaviorally meaningful changes occur. The type B photoreceptor, hippocampus, and the lobule HVI area of the cerebellar cortex will provide the main foci for this work.

We plan to identify the mechanisms leading to the observed changes in protein phosphorylation specific to learning in *Hermisenda*, the rabbit, and the rat, i.e., involving changes of adenylate cyclase, phosphodiesterase, protein kinase and/or phosphatase activities. Other mechanisms of posttranslational modification will also be explored. We also will study the synthesis and modification of gene products in these nervous systems by means of molecular biologic techniques. Studies will also be continued to isolate the proteins which undergo transformation during learning. The relationship of such proteins to the structure of membrane channels as well as their regulation will continue to be investigated.

4) Analytic tools (via the use of modern molecular biology) such as monoclonal antibodies will be used to scan large neuronal arrays in mammalian brain slices. "Images" of such arrays in these slices will then be reconstructed to yield three-dimensional representations of conditioning-specific shifts of neuronal system metabolism. Such "images" represent an extension of methodology already proven to be highly effective (with, for example, radioactive phorbol ester) for assaying memory-specific functions of large neuronal arrays.

5) Mathematical models of *Hermisenda* classical conditioning will be generated to provide quantitative description of membrane ionic currents, neural systems and behavior. Mathematical transformations will be derived to allow interfacing of quantitative descriptions at these different levels of biological complexity. Artificial systems will be designed based on *Hermisenda* models (as well as confirmed generalities to vertebrate brain slices). Successful creation of such artificial systems will not only have inherent practical value, but will also serve to indicate how complex biological systems (not amenable to comprehensive description) may function.

6) Differential absorption spectrophotometry will be further utilized for localizing intracellular fluctuations of cytosolic Ca^{2+} as they occur during different phases of the learning process. Cytochemical measurements within individual neurons will be further applied to reveal neurochemical means of amplifying the Ca^{2+} -dependent modulation of the channels during associative learning.

7) Behavioral experiments will be continued to further determine the comparability of the *Hermisenda* associative learning model to associative learning defined for more evolved species.

- 8) Long-term effects of conditioning, olfactory learning, and spatial maze learning will be investigated. Neural and behavioral changes which last days and weeks will be compared to those already determined. The interaction of developmental processes with these longer-term changes will also be studied.
- 9) The relationship of K⁺ channel changes with learning to changes of neuronal architecture will be studied with confocal microscopy. Molecular steps required for both structural and channel changes will be identified.
- 10) A voltage sensitive dye apparatus has been specially developed and built for multisite optical recording of the visual-vestibular network in *Hermisenda*. We will characterize the role of this small network in the processing of sensory information, and its role in associative memory mechanisms.
- 11) Molecular biologic analysis of memory-specific genetic loci will be pursued using recombinant DNA techniques, PCR and genetically defined strains of lab-reared animals.
- 12) Genetic linkage (i.e., chromosomal mapping) of Alzheimer's genes with genes for memory-specific G proteins (e.g., cp20) as well as isozymes of PKC will be investigated.
- 13) Biophysical and molecular analyses of Alzheimer's and control cells (e.g., fibroblasts, olfactory neurons, lymphocytes) will be continued to assess second messenger systems (e.g., for PKC) and particularly their regulation of K⁺ channel function.
- 14) The generality of cellular principles of learning and development determined for relatively "simple" neural systems and the behavior they control will continue to be examined in more evolved species. Brain slices will continue to be obtained from rabbits and rats exposed to conditioning and pattern recognition procedures, and further analyzed as possible sites for encoding and storage of learning experience. Ultimately, mechanisms common to organisms with a wide range of evolutionary diversity and complexity may contribute to understanding the human nervous system and may motivate clinical approaches.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS02151-18 LMCN
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Memory Storage in Neural Networks		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: D. L. Alkon Medical Officer LMCN NINDS Others: NINDS: T Nelson, Chemist; D Lester, Vis Assoc; C Collin, Vis Assoc; R Etcheberrigaray, Vis Assoc; L Wang, Vis Assoc; G Adam, Vis Assoc; S Moshiaich, Vis Assoc; B Schreurs, Senior Staff Fellow; J Olds, Senior Staff Fellow; JV Sanchez-Andres, Vis Fellow; E Ito, Vis Fellow; CJ Lee, Vis Fellow; Y-F Han, Vis Fellow; E Maduh, Vis Fellow; D McPhie, IRTA Fellow; J Schachter, Staff Fellow; L Matzel, Spec Volunteer; AM Craig, Spec Volunteer; K Kusuzaki, Spec Volunteer; K Oka, Spec Volunteer.		
COOPERATING NITS (if any) Marine Biological Laboratory, Woods Hole, MA 02543 (A. Kuzirian); California Institute of Technology (C. Chen); Medical Research Council, Canada (B. Bank)		
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology, BNP, DIR, NINDS		
SECTION Neural Systems Section		
INSTITUTE AND LOCATION Park Building, Room 431 and Building 9, Room 1W125, NINDS, Bethesda, Maryland 20892		
TOTAL STAFF- <div style="display: flex; justify-content: space-between; width: 100%;"> 10.0 </div>	PROFESSIONAL: <div style="display: flex; justify-content: space-between; width: 100%;"> 9.0 </div>	OTHER: <div style="display: flex; justify-content: space-between; width: 100%;"> 1.0 </div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-around; align-items: flex-start;"> <div style="text-align: center;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="text-align: center;"> <input type="checkbox"/> (b) Human tissues </div> <div style="text-align: center;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The principal objective of the program is to define molecular and biophysical mechanisms of <u>learning and memory</u>. Emphasis is placed on learning and memory which can be related to human cognition. Ultimate goals of such research are to arrive at clinically meaningful interventions and to design and construct artificial intelligence which has advanced learning and memory capabilities. With human cognitive function as the principal frame of reference, the research focuses on associative processes (such as Pavlovian conditioning) rather than non-associative behavioral modifications (such as sensory adaptation, habituation, arousal, and sensitization). The <u>biological basis of learning and memory</u> is of interest at several levels of complexity: <u>behavior, neuronal systems, neuronal architecture and membranes and molecular transformations</u>. To reconstruct the physiology involved (and to model it in artificial contexts) it is necessary to use both "simple system" preparations such as the nudibranch mollusc <i>Hermisenda crassicornis</i> as well as "complex system" preparations such as rabbits and rats. The molluscan work thus far has yielded the first unequivocal biological record of an associative memory. This record consists of persistent transformations of specific ionic channels. Because these records have been found within the membranes of <u>identified single neurons</u> it has proven possible to define biochemical pathways which regulate such long-term membrane modifications as well as to analyze how this biological memory record is expressed by the integrative functions of an entire neuronal system. The work on the vertebrate brain offers two essential opportunities. First, the generality of mechanisms determined for much less evolved species can be tested. Remarkably, the same ionic channel transformations have been shown in our program to record associative memory in the rabbit as were found in <i>Hermisenda</i>. Rabbit and now rat neural systems have also provided sufficient quantities of tissue so that conditioning-specific alterations of critical enzymatic (e.g., <u>protein kinase C</u>) pathways which control membrane excitability have recently been demonstrated. Furthermore, identical G protein substrates which regulate similar K⁺ channels undergo memory-specific modification in mollusc and mammals. Such biophysical and molecular parallels in mechanisms of memory storage suggest the possibility of general cellular principles of memory storage with significance for human physiology and pathophysiology as well. </p>		
23-LMCN/BNP/DIR		

ANNUAL REPORT
October 1, 1991 through September 30, 1992
Receptor Biochemistry and Molecular Biology Section,
Office of the Director
Basic Neuroscience Program, DIR
National Institute of Neurological Disorders and Stroke
J. Craig Venter, Ph.D., Chief

The Section of Receptor Biochemistry and Molecular Biology (RBMB) conducts research on characterizing the expressed gene content of the human brain, and on gene super-families of neurotransmitter receptors, and on the study of genomic structure of regions potentially involved in neurological diseases. A highly automated system has been developed in the Section to perform rapid DNA sequencing and analysis of the results.

The Section has made major advances in the sequencing and analysis of expressed sequence tags (ESTs), or partial cDNA sequences. As many as half of the over 50,000 human genes are believed to be expressed in brain. While sequencing the human genome is expected to take over 15-20 years, sequencing a large number of clones can readily provide coding sequence data on genes expressed in specific tissues. Nearly 8000 ESTs have been isolated from several human brain libraries, sequenced and analyzed. Nearly 80% of the genes isolated had not been previously identified. An additional 6% are newly identified human genes, homologues of which had previously been identified in other species. Further characterization of potentially interesting clones includes chromosomal localization, examination of tissue distribution and evolutionary conservation.

A similar project has been under way with clones from the nematode *Caenorhabditis elegans*. The goal of this program is to isolate, partially sequence and map genes from *C. elegans* as a model system to study the function of genes responsible for human disease. We have partially sequenced over 2500 cDNA clones from this organism. Over 30 of these genes have been physically localized on the *C. elegans* genomic map. The genes identified include homologs to a large number of genes important in human neural function or human diseases.

We have studied the genomic organization of the neurofibromatosis (NF1) gene from chromosome 17, in collaboration with Dr. Francis Collins of the University of Michigan. Several exons and Alu repeats have been localized in the genomic sequence.

The two human chromosomal sequencing projects initiated in 1990 and described in the 1991 Annual Report have been completed, and the results published. Three cosmids from chromosome 4p16.3, the Huntington's

disease region, and three cosmids from chromosome 19q13.3, distal to the ERCC1 gene, were sequenced. This project was the first in which human chromosomal DNA of unknown gene content was sequenced and analyzed to discover new genes. As such, it is a test of the sequencing and analysis methodologies proposed for the Human Genome Program.

We have applied the technologies we have developed in our megabase sequencing projects to the sequencing of the 178 kb smallpox genome. All stocks of the virus are scheduled for destruction in 1993, and we are nearly finished determining the sequence of this virus so a record of its genome structure will remain.

The Section of Receptor Biochemistry and Molecular Biology also runs the NINDS DNA facility. This facility synthesizes oligonucleotides for Institute laboratories and branches. During FY92 the DNA synthesis facility has synthesized 2467 oligonucleotides for the Institute.

Dr. Venter and the majority of his staff left the NIH in August, 1992 to set up The Institute for Genomic Research in Gaithersburg, Maryland. All of the projects listed in this report have been transferred to The Institute for Genomic Research.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS02806-03 RBMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Brain cDNA Project

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.C. Venter, Ph.D., Chief, RBMB, OD, NINDS; Others (RBMB, OD, NINDS):
M. D. Adams, Ph.D., Staff Fellow; W.R. McCombie, Ph.D., Sr. Staff Fellow;
A.R. Kerlavage, Ph.D., Sr. Staff Fellow; M. Dubnick, Ph.D., Sr. Staff Fellow;
C. Fields, Ph.D., Special Expert; J.M. Kelley, M.S., Microbiologist; T.R.
Utterback, Microbiologist.

COOPERATING UNITS (if any)

J. Powell, CSL, DCRT; J.C. Kelley, CSL, DCRT

LAB/BRANCH

Receptor Biochemistry and Molecular Biology Section, OD, NINDS

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF YEARS:

4.5

PROFESSIONAL:

2.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to isolate, sequence, and characterize the genes expressed in human brain. As many as half of the almost 50,000 human genes are believed to be expressed in the brain. While sequencing half of the human genetic material is expected to take over a decade, and coding regions in the resultant genomic sequence may not be clearly discerned, sequencing a large number of cDNA clones can readily provide coding sequence data. We are building a large library of sequences of human brain cDNA clones. The availability of a broad-based library of cDNA sequences will facilitate identification of coding regions in genomic sequences as well as providing a starting point for individual cloning projects. A variety of approaches are being used to select brain-specific clones and to eliminate highly represented sequences. Over eight thousand human brain genes have been identified by this method. Computer analysis of DNA and predicted protein sequences were performed to search for the presence of conserved primary structure motifs and relationships to previously sequenced genes. It was found that over half of the sequences represented new genes with no detectable similarity to previously sequenced genes. An additional percentage represent the human homolog of genes that have been sequenced in other organisms. Further characterization of several interesting clones with potential roles in neural development is currently in progress and will include chromosome localization, examination of tissue distribution of expression, functional analysis, and evolutionary conservation.

This project has been transferred to The Institute for Genomic Research.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02754-05 RBMB

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Megabase DNA Sequencing

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: JC Venter, Ph.D., Chief, RBMS, OD, NINDS

Others (RBMS, OD, NINDS): WR McCombie, Ph.D., Sr. Staff Fellow; A Martin-Gallardo Ph.D., Sr. Staff Fellow; AR Kerlavage, Ph.D., Sr. Staff Fellow;

M Dubnick, Ph.D., Staff Fellow; M FitzGerald, BA., Biologist; JM Kelley, B.S., Biologist; L Liu, Ph.D., Special Volunteer, T Utterback, B.A., Special Volunteer.

COOPERATING UNITS (if any)

J Powell, CSL, DCRT; JC Kelley, CSL, DCRT; F Collins, M.D., Ph.D., University of Michigan; B Mahy, Centers of Disease Control

LAB/BRANCH

Receptor Biochemistry and Molecular Biology Section, OD, NINDS

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INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The goal of this project is to isolate and sequence fragments of genomic DNA that encode neurotransmitter receptors or are in regions associated with neurologic diseases. Such sequences can be used as a starting point to determine the structure and regulation of these neurotransmitters and aid in the precise localization and identification of the lesion responsible for the genetic disease. In order to accomplish these goals, we have continued to develop new laboratory procedures and test new automation to rapidly increase the rate at which we are able to sequence and analyse genomic DNA. We have continued the analysis of three cosmids we sequenced from chromosome 4 that were derived from the 2.2 million base region thought to contain the Huntington's disease gene. We have determined the complete structure of one of the genes contained in this region. We have also identified 72 polymorphisms in this sequence which can be used to further refine the location of the Huntington's disease gene. The sequence of a Drosophila octopamine receptor gene has been nearly completed and the promoter for that gene isolated. We have also, in a collaborative effort, sequenced portions of the gene responsible for neurofibromatosis. In addition we have nearly completed sequencing of the smallpox variola virus.

This project has been transferred to The Institute for Genomic Research.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS02837-02 RBMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Neurological Genes in *Caenorhabditis elegans*.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.W.R. McCombie, Ph.D., Sr. Staff Fellow, RBMBS, OD, BNP, DIR, NINDS
Others (RBMBS, OD, NINDS): J.M. Kelley, M.S., Microbiologist; M. Fitzgerald, B.A., Biologist; C.Fields, Ph.D., Special Volunteer; Lisa Aubin, B.A., Biologist; A.R. Kerlavage, Ph.D., Sr. Staff Fellow; M. Kahn, Stay in School, M. Dubnick, Ph.D., Sr. Staff Fellow; M. Adams, Ph.D., Staff Fellow; J. C. Venter, Ph.D., Chief, RBMBS

COOPERATING UNITS (if any)

Alan Coulson, LMB, MRC, Cambridge, U.K.

LAB/BRANCH Receptor Biochemistry and Molecular Biology Section, Office of the Director, BNP, DIR, NINDS

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this program is to isolate, partially sequence and map the genes expressed in the nematode *Caenorhabditis elegans*. We have partially sequenced over 2500 cDNA clones from this organism. Over 30 of these genes have been physically localized on the *C. elegans* genomic map. The genes identified include homologs to a large number of genes important in human neural function or human diseases. These include acetylcholinesterase, a serotonin receptor, a potassium channel, the tat-binding protein, and prohibitin, among others.

This project has been transferred to The Institute for Genomic Research.

ANNUAL REPORT

October 1, 1991 through September 30, 1992

Laboratory of Biophysics
Basic Neurosciences Program, DIR
National Institute of Neurological Disorders and Stroke
Gerald Ehrenstein, Ph.D., Acting Chief

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Annual Report

October 1, 1991 through September 30, 1992

Laboratory of Biophysics

National Institute of Neurological Disorders and Stroke

Gerald Ehrenstein, Ph.D., Acting Chief

We are currently studying a number of ionic channels, with particular emphasis on the relationship between the properties of the channels and their physiological roles. Channels being studied include calcium channels, calcium-activated channels, potassium channels, sodium channels, and nonselective cation channels. These channels play a significant role in signalling in the nervous system, in the firing pattern of heart cells, in the increase in intracellular calcium associated with the secretion of neurotransmitters and hormones, and probably in the secretion process, itself. Determination of the electrical, chemical, and pharmacological characteristics of the channels provide information that can be used to test models of the basic mechanisms of the physiological processes being studied.

One of the processes we are studying is the entrance of calcium ions into cells that secrete neurotransmitters and hormones. The resultant change in intracellular calcium concentration is a crucial factor in controlling the amount of secretion. There are two systems of particular interest that we have been studying. One is the vertebrate presynaptic calyx of the chick ciliary ganglion, which secretes the neurotransmitter acetylcholine (ACh). The other is the bovine parathyroid cell, which secretes parathyroid hormone (PTH). The former has provided us an opportunity to record for the first time the calcium currents in a vertebrate presynaptic nerve terminal. The latter is a cell with the unusual property that increased intracellular calcium results in a decrease in secretion. Thus, studies of these systems are likely to lead to understanding of very basic mechanisms.

The calyx synapse preparation has now been used to record presynaptic calcium and potassium channels at the single-channel level. Calcium channels were found on the inner face of the calyx, consistent with their putative role in allowing the entrance of calcium ions near the release sites. These channels have a single-channel conductance of about 13 pS, and can be highly clustered. The success of this project in measuring these channels provides an opportunity to extend this technique to the measurement of other channels and also to the recording of neurotransmitter release. This should allow us to determine the relationship between channel opening and transmitter release. We are

also trying to apply similar techniques to examine the hippocampal mossy fiber nerve terminal. This is of particular interest because it would extend these measurements to the mammalian CNS and also because the mossy fiber synapse exhibits long-term potentiation.

Unlike secretion from most secretory cells, secretion of PTH from parathyroid cells decreases when intracellular calcium concentration increases. Since PTH acts to increase extracellular calcium and increased extracellular calcium acts to increase intracellular calcium, the anomalous PTH secretion results in a negative feedback system that serves to keep the calcium concentration stable. A key factor governing the relationship between intracellular and extracellular calcium concentration is the steady-state calcium influx. In order to determine the magnitude of this influx, we used single-channel and whole-cell patch clamping to determine the properties of the pathway for steady-state calcium current. We found an unusual calcium channel, one that is not sensitive to membrane potential and does not require receptor binding. For a typical parathyroid cell under physiological conditions, this channel has a single-channel conductance of about 0.6 pS, corresponding to a steady-state calcium current of about 0.9 picoamperes per cell.

The properties of these calcium channels have several implications regarding the functioning of parathyroid cells. It has previously been observed that there is decreased intracellular calcium concentration with increasing membrane depolarization, and this can be accounted for by the voltage independence of these channels. In terms of cell function, the voltage independence allows secretion of parathyroid hormone to be sensitive to the extracellular calcium concentration without being unduly sensitive to membrane potential.

In the steady-state, the calcium current and the calcium concentration are constant, and it is of interest to estimate the density of calcium pumps that are required to maintain the steady-state. According to our calculations, the density required is about 100 pumps per square micrometer per second, about five times the density of pumps in the erythrocyte, and about twice the density in the hen shell gland. Thus, despite the presence of a pathway for steady-state calcium influx, the density of calcium pumps required is not unduly large. One reason for this is that the calcium channels are open only about 8% of the time.

We have also been studying sodium and potassium channels in squid giant axons, and, in particular, the process by which these channels are inserted into the plasma membrane. It is known that the channels are carried by axoplasmic organelles, and we have

now separated out an organelle of about 50 nm diameter that contains both sodium and potassium channels. We have studied the properties of these channels by inserting them into lipid bilayers, and found, in addition to channels that are identical to known channels in squid giant axons, voltage-independent potassium channels that may play a role in determining the resting potential. An interesting feature of this organelle is that it contains a much higher density of channels than does the plasma membrane, thus affording an improved opportunity for structural analysis of the channels.

We have also been studying the release of superoxide radical from microglia cells. This release is part of the response triggered by foreign or unwanted cells, and results in their oxidation and destruction. We have previously compared the release of superoxide from microglia of mice that are models for Downs syndrome with the release of superoxide from microglia of normal littermates. We now have measured the effect of high potassium on the release of superoxide. We found that depolarization of microglia by high extracellular potassium results in increased superoxide release in the presence of the activator phorbol myristate acetate (PMA). We also found that the microglia contain L-type calcium channels that open and admit calcium ions when the cell is depolarized, suggesting that the combined action of PMA and calcium stimulates superoxide release. This would provide an effective means of eliminating damaged brain cells, since the damaged cells would release potassium into the extracellular space and the depolarization would increase the entry of calcium ions into the microglia. This would increase the release of superoxide and thus tend to eliminate the damaged cells. We have also found that microglia release nitric acid (NO) in response to activation by lipopolysaccharide and by gamma interferon. The combined effect of superoxide and NO might be a more potent oxidation, perhaps by means of the hydroxyl radical.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02608-08 LB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Comparative Aspects of Ionic Conductances in Nerve and Heart Cell Membranes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	John R. Clay, Ph.D.	Staff Physicist LB, NINDS
Others:	Vijay Kowtha, Ph.D.	NRC Fellow LB, NINDS
	Keith E. Krebs, Ph.D.	Senior Staff Fellow LB, NINDS
COOPERATING UNITS (if any) McGill University (A. Shrier), Marine Biology Lab (A. Kuzirian), Univ. of West Virginia (W. Wonderlin)		
LAB/BRANCH Laboratory of Biophysics, BNP, DIR		
SECTION		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF-YEARS:	2.4	PROFESSIONAL: 2.3 OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project is concerned with a comparative analysis of <u>ion channels</u> in <u>nerve</u> and heart cell membranes and the relationship of these channels to excitability in both preparations. During the past year, one of the primary experimental preparations has been the <u>axoplasm</u> from <u>squid giant axons</u>. The axoplasm has been previously shown to contain <u>organelles</u> which, in turn, contain potassium and sodium ion channels that are virtually identical to the respective ion channels underlying excitability in the axolemma. Those organelles appear to fuse with the axonal membrane, thereby replenishing these channels in the axon. This project has focused on a follow-up to these original observations. One major finding has been that the organelles can be separated on the basis of size using <u>control-pore-size</u>, glass bead <u>chromatography</u>. One major organelle fraction obtained with this chromatography technique lies near the 50 nm size point of the glass bead column, which probably corresponds to <u>anterograde</u> organelles. These organelles were also found to contain sodium and potassium ion channels based on recordings of electric currents from channels incorporated in artificial <u>lipid bilayers</u>. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02709-07 LB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Secretion of Neurotransmitters and Hormones		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: G. Ehrenstein, Ph.D. Others: K. Krebs, Ph.D. M. Jia, M.D. M. Li, M.D. A. Mbuyi-Kalala, Ph.D.	Research Physicist Senior Staff Fellow Visiting Associate Visiting Associate Visiting Associate	LB, NINDS LB, NINDS LB, NINDS LB, NINDS LB, NINDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Biophysics, BNP, DIR		
SECTION		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF-YEARS: 4.6	PROFESSIONAL: 4.2	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)		
<p>We have made direct measurements of the properties of the <u>channels</u> through which <u>calcium</u> enters <u>parathyroid cells</u> under normal physiological conditions. These channels are <u>voltage-independent</u>, and their single-channel conductance at the physiological calcium concentration is about 0.6 pS.</p> <p>We are developing an improved assay for <u>parathyroid hormone</u> (PTH) secretion in order to determine how the <u>secretion</u> of PTH is affected by different <u>divalent cations</u>, and how it is affected by gradients of <u>osmotic pressure</u> across different components of the parathyroid cell.</p> <p>We are using two complementary techniques to determine the properties of ionic channels in the membranes of <u>secretory vesicles</u> of the <u>neurohypophysis</u>. One technique involves measurement of the channels after they have been incorporated into <u>lipid bilayers</u>, and the other technique involves immunopurification of <u>mRNA</u> for the channels from <u>polysomes</u> and expression of this mRNA in frog oocytes.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02609-09 LB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanism of Egg Activation Following Fertilization		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: G. Ehrenstein, Ph.D. Others: K. H. Iwasa, Ph.D. P. Smolen, Ph.D.	Research Physicist Special Expert NRC Research Associate	LB, NINDS LCB, NIDCD MR, NIDDK
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Biophysics, BNP, DIR		
SECTION		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p> We have previously found that at the time of <u>fertilization</u>, a single <u>sea urchin spermatozoon</u> contains at least 2×10^{-18} moles of <u>inositol trisphosphate</u>, a <u>second messenger</u> known to cause release of <u>calcium</u> from intracellular organelles. We have also calculated the relative <u>activation</u> efficiencies of direct insertion by the spermatozoon and insertion by injection, and found that direct insertion by the spermatozoon is about three times more efficient than insertion by injection. Since the quantity of inositol trisphosphate required to initiate activation of <u>sea urchin eggs</u> by injection is known to be about 3×10^{-18} moles, it is likely that a single spermatozoon can insert enough inositol trisphosphate into the egg to initiate activation, and thus, that inositol trisphosphate may act as a <u>primary messenger</u> in the fertilization process. We are planning to test this hypothesis by loading <u>liposomes</u> with appropriate amounts of inositol trisphosphate, and observe whether the liposomes can activate eggs. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02606-09 LB												
PERIOD COVERED October 1, 1991 through September 30, 1992														
TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.) Calcium Channels in Vertebrate Nerve Terminals														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI :</td> <td style="width: 40%;">E. F. Stanley, Ph.D.</td> <td style="width: 30%;">Staff Physiologist</td> <td style="width: 20%;">LB, NINDS</td> </tr> <tr> <td>Other:</td> <td>Xaio Ping Sun, Ph.D.</td> <td>Visiting Fellow</td> <td>LB, NINDS</td> </tr> <tr> <td></td> <td>Henry Markram, Ph.D.</td> <td>IRTA Fellow</td> <td>LB, NINDS</td> </tr> </table>			PI :	E. F. Stanley, Ph.D.	Staff Physiologist	LB, NINDS	Other:	Xaio Ping Sun, Ph.D.	Visiting Fellow	LB, NINDS		Henry Markram, Ph.D.	IRTA Fellow	LB, NINDS
PI :	E. F. Stanley, Ph.D.	Staff Physiologist	LB, NINDS											
Other:	Xaio Ping Sun, Ph.D.	Visiting Fellow	LB, NINDS											
	Henry Markram, Ph.D.	IRTA Fellow	LB, NINDS											
COOPERATING UNITS (if any)														
LAB/BRANCH Laboratory of Biophysics, BNP, DIR														
SECTION														
INSTITUTE AND LOCATION														
TOTAL STAFF-YEARS: 2.7	PROFESSIONAL: 2.5	OTHER: 0.2												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews					
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither												
<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Calcium-dependent release of neurotransmitters</u> is a crucial step in virtually all aspects of central and peripheral neuron function. The study of transmitter release has been limited, however, by the lack of suitable experimental preparations. Last year we reported the first recording of <u>calcium currents</u> in a vertebrate presynaptic nerve terminal using the calyx synapse of the chick ciliary ganglion. We have extended this study by modifying this technique to allow the recording of presynaptic calcium currents at the single channel level. These channels have a single channel conductance in the 13 pS range, and can be highly clustered. This report is the first description of calcium channel properties at the <u>single channel level</u> at any <u>presynaptic nerve terminal</u>. We will use this presynaptic nerve terminal preparation to search for other presynaptic ion channel types, both ligand-and voltage-gated. In addition, we are developing a technique to carry out patch-clamp recordings from a mammalian central nervous system presynaptic nerve terminal, i.e., the mossy fiber terminal in the hippocampus. </p>														

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02218-17 LB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sources and Effects of Reactive Oxygen Intermediates in the Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. L. Gilbert, Ph.D.	Research Physiologist	NINDS, LB
Others:	Min Jia, M.D.	Visiting Associate	NINDS, LB
	Minxu Li, M.D.	Visiting Associate	NINDS, LB

COOPERATING UNITS (if any)

NIMH, NIH (C.C. Chiueh), CNS, NINDS (K. Bankiewicz, D. Lieberman) Georgetown Univ., DC (C.A. Colton, G. Thomas, J. Keri, F. Pagan) Howard Univ., DC (J. Stewart).

LAB/BRANCH

Laboratory of Biophysics, BNP, DIR

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:

1.7

PROFESSIONAL:

1.4

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experiments have been performed on microglia, the resident macrophage in the central nervous system (CNS) cultured from rat cerebral cortex. We have previously shown that these activated cells produce the superoxide radical anion, a reactive oxygen species (ROS). ROS also includes the hydroxyl radical and hydrogen peroxide. Earlier, we found that calcium ions can enter microglial cells when these cells are hyperpolarized. We have continued our studies using the patch-clamp technique in cultured microglial cells on the L-type voltage-dependent calcium channels which open when these cells become depolarized. These channels may play a role in our finding that potassium can modulate the production of the superoxide radical anion from microglial cells. We are also continuing our studies with iron toxicity and its relationship to the production of the highly toxic hydroxyl radical. Survival of dopaminergic neurons grown in the presence of glutathione peroxidase show more diffuse neuronal branching, indicating that hydrogen peroxide is involved.

ANNUAL REPORT

October 1, 1991 through September 30, 1992

Laboratory of Central Nervous System Studies

National Institute of Neurological Disorders and Stroke

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ANNUAL REPORT
Laboratory of Central Nervous System Studies
October 1, 1991-September 30, 1992
D. Carleton Gajdusek, M.D., Chief

Viliuisk encephalomyelitis (VE) has become one of our major targets of research during this year, and will loom larger in our future work. We have been involved for 30 years in this problem of a fatal chronic or subacute basilar encephalitis which affects the Sakha (Iakut) people of northern Russian Siberia. The cause of this chronic brain infection, which is spreading outside the original endemic focus, has not been found. With an unique clinical spectrum and a new unique neuropathology indicating a massive chronic inflammatory response in microfocal lesions, with ongoing fresh lesions even decades after the onset, VE presents a major challenge to all studies on the pathogenesis of chronic brain infections with important implications for HIV (AIDS) encephalopathy and HTLV-I myeloneuropathy, subacute sclerosing panencephalitis, progressive multifocal leukoencephalopathy, and even central nervous system (CNS) syphilis and visceral larva migrans and mycobacterial infections. In view of the clear low-grade communicable spread into new populations, it would be as foolish to disregard this disease, as it was to disregard the African cases of acquired immunodeficiency syndromes in the 1950s, 1960s and 1970s which led to the AIDS pandemic.

We have published a definitive clinical and epidemiologic survey of 20 years of study of VE in the October, 1992 issue of *Brain*, and additional major reports to alert the world neurologic community to the ongoing problem will be published during the coming year, including a third expanded edition of our VE bibliography.

Three members of our laboratory have recently returned from a month-long expedition to the Sakha Republic, with CSF, sera and leucocytes in transport media, as well as frozen in liquid nitrogen or on dry ice, and also with DNA specimens from many VE patients. We have already excluded dozens of possible groups of pathogens by serologic testing, and these new specimens will be examined using both classic and molecular biology techniques.

Part of the Sakha (Iakut) Siberian VE problem involves the differential diagnosis of VE from a second intriguing neurologic problem in the Iakut people of the largest kindreds of Pierre-Marie (P-M) hereditary olivopontocerebellar degeneration behaving as an autosomal dominant genetic trait. It occurs largely in the Iakut people in the northern Indegirka and Aldan River Valleys. We have reported these foci in *Annals of Neurology*, 1989. We have now seen dozens of new patients from whom, and from whose family members, we have obtained sufficient DNA to enable us to find the responsible gene mutation, which can then be used as a molecular genetic probe to ascertain the diagnosis. This will help greatly in distinguishing the two diseases (VE from P-M) in populations of Iakut in the Aldan and Viliuisk River Valleys where both diseases overlap.

Our discovery of a paleo-Melanesian strain of human T lymphotropic virus type I (HTLV-I) in remote populations in Papua New Guinea and the Solomon Islands, east of Wallace's Line, where no monkeys or apes have ever existed, has greatly changed our views of the possible origins and evolution of this human retrovirus. Detailed sequencing of the major viral gene regions indicates that the Melanesian variants of HTLV-I are more closely related to the Asian subtypes of simian T lymphotropic virus type I and to HTLV-II than are the Cosmopolitan strains of HTLV-I. Paleo-Melanesian strains of HTLV-I are also found in some Aboriginal tribes in central and northern Australia. This leads us to predict that the common ancestor of the Austro-Melanesian HTLV-I strains evolved somewhere in the Southeast Asian landmass (Sunda) during the Pleistocene epoch when land bridges connected New Guinea and Australia and formed the Sahul continent. We are pursuing this important lead to unravel the evolution and early dissemination of this human retrovirus. Also, since we have found HTLV-II infection to be very prevalent in many scattered tribes of Amerindians in South America, we are searching for the presence of HTLV-II in trans-Siberian northern peoples related in the distant past to the Amerindian ancestors.

Our primary area of inquiry continues to be the elucidation of the pathogenesis of dementing brain amyloidoses, both the transmissible type [such as kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler syndrome (GSS), scrapie, and bovine spongiform encephalopathy (BSE), caused by infectious amyloidoses] and the nontransmissible type [such as normal aging, Alzheimer's disease (AD), and Down's syndrome]. We are now concentrating on the protein chemistry and crystallography of the configurational change of host precursor protein to the infectious amyloid form both *in vivo* and *in vitro*. Our program continues to characterize the molecular basis for the genetic control of *de novo* generation of infectious amyloid proteins from host precursors in kuru, CJD, GSS, scrapie and BSE.

We are also concentrating on the phenomenon of nucleation with the induction of patterned configurational change. Many proteins in the amyloid β -pleated configuration induce other amyloidogenic molecules to form amyloid by this process, which we call "induction of configurational change" and which others call "instructional amyloidogenesis". We have for long believed that the amyloid-enhancing factors (amyloid-augmenting factors) are really scrapie-like agents nucleating the configurational change to amyloid, as do amyloid fibers themselves (fibril amyloid-enhancing factors). The polymer chemistry of nucleation and of fibril polymerization has much to teach us in this field which will be applicable to studies of amyloidogenesis in AD, aging brain, and in the transmissible brain amyloidoses (kuru-CJD-GSS-scrapie-TME-BSE).

Many other laboratories are now pursuing the problem, first phrased and delineated by us, of the unconventional viruses which are replicating proteins, to determine how the amyloid precursor protein is converted to an infectious form by configurational changes in the tertiary and quaternary structure of the normal precursor. On inoculation of susceptible hosts, the CJD or scrapie amyloid monomer autonucleates and autopatterns this conversion of the normal, noninfectious host precursor into the infectious form. We believe that most sporadic cases of CJD arise by *de novo* spontaneous conversion of the normal precursor to the infectious form, a rare event occurring at the frequency of one per million persons per year (the annual incidence of CJD throughout the world). In the familial forms of CJD and GSS in which the occurrence is an autosomal dominant trait, we have found that each family has one of several different mutations, 6 causing a single amino acid change and the other causing the insertion of 2, 5, 6, 7, 8, 9 or 10 octapeptide repeats. Each mutation causes a million-fold increased probability of the spontaneous configurational change to an infectious polypeptide, and appears as an autosomal dominant trait. Thus, the behavior of the transmissible brain amyloidoses parallels completely that of the transthyretin amyloidoses causing familial amyloidotic polyneuropathy, in which there are more than 30 point mutations, each of which enormously increases the likelihood of a configurational change of prealbumin to an amyloid in different families.

Our laboratory has had a series of discoveries in rapid succession of several point mutations causing single amino acid substitutions which lead to distinct different forms of CJD or GSS differing in clinical course, incubation period, duration, EEG changes, and distribution of lesions and presence of amyloid plaques. Incubation period on first passage in primate hosts may vary for different mutations. Yet, on second passage into primate hosts, the differences apparently disappear. Our discovery that the codon 200 point mutation (glutamic acid to lysine) causes the CJD in the two high-incidence foci of CJD in the Orava and Lucenec regions of Slovakia) has been followed by our demonstration that this same point mutation is linked to CJD cases in Eastern Europe from Poland through Greece, and even in cases in the United States and in South Americans of Eastern European Slavic origin. This has led to our quickly discovering that this same point mutation underlies the high incidence of CJD in Sephardic Jews of Libyan origin, both in immigrants to Israel and Israeli-born. Furthermore, we have found this same mutation in Sephardic Jews with CJD from Tunis and Greece and thus, it is a circum-Mediterranean Jewish trait as well as of Eastern European Slavs. Since the Jews with the Arabs moved across Northern Africa to the Iberian Peninsula, and, in the late 15th century, fled as Sephardic Jews from Spain to Greece and elsewhere in the Eastern Mediterranean, this codon 200 mutation has proved to be a marker of the "Wandering Jew of the Diaspora". We have shown that this codon mutation 200 also underlies the high incidence of familial CJD in Chile. We have also traced throughout Europe and the Americas a codon 178 mutation (aspartic acid to asparagine) which causes a rapidly progressive form of CJD.

Fortunately, from our CJD transmission work spanning 30 years we know that most of the mutations, including the octapeptide repeat inserts, produce infectious amyloids which are transmissible to susceptible laboratory animals, some with incubation periods lasting more than 10 years. We have transmissions in monkeys or chimpanzees from CJD cases with each of the point mutations or inserts, and the affected primates produce infectious amyloids which do not contain these point mutations or insertions.

The possibility that a synthetic polypeptide containing the precursor protein sequence with codons 102, 178, and 200 mutations, or the octapeptide inserts themselves, might be infectious and serve to nucleate the autopatterned configurational change in the host precursor protein to its infectious form is being investigated by inoculation of many species with such synthetic polypeptide. Finally, we are also investigating the possible infectiousness of baculovirus-expressed peptides of the human CJD amyloid containing these and other mutations.

We continue to insist that normal brain aging and Alzheimer's disease (AD) are the same type of nontransmissible amyloidosis, with different speeds of conversion of the β A4 amyloid precursor protein to amyloid fibrils. Environmental, nongenetic, probably toxic causes surely influence the amyloid conversion in sporadic AD. However, in some families with familial AD, there is one of several point mutations on the β A4 amyloid precursor protein at codon 717 (valine to isoleucine, phenylalanine, or glycine), and in families with Dutch hereditary cerebral hemorrhage, a different point mutation at codon 613 (glutamic acid to glutamine). In the latter, the point mutation is expressed only in endothelial cells producing β -amyloid fibrils around vessels, but no amyloid plaque cores or amyloid in neurofibrillary tangles. We were the first to characterize the gene for this aging brain amyloid precursor protein (β A4 protein), to locate it on chromosome 21 of man and 16 of mouse, and to show its high evolutionary conservation. This gene is identical to that for an excreted rapidly turned-over protein which specifically binds to the subunit of nerve growth factor and many other serine proteases and cell modulators which contain such serine protease as binding regions. This important negative feedback control loop may explain the rapid synthesis, short half-life and wide distribution in neurons of this brain amyloid precursor. Our work showing that the gene was expressed in several alternatively spliced forms, with and without a 57 bp or 76 bp insert which specifies a serine protease inhibitor, now fits well with the identity of protease nexin II and the alternatively spliced forms of our amyloid precursor protein containing the serine protease inserts.

Our studies of amyloid β -protein mRNA expression in different brain cells of normal juveniles, aging brain and AD brains have revealed that all neurons which develop neurofibrillary tangles (NFT) and are most vulnerable to loss in aging, and in AD, express a very high level of turned-on message. However, not all cells with high levels of amyloid β -protein mRNA develop NFT, and thus its high expression appears to be a necessary, but not a sufficient, condition for NFT formation. Interestingly, in Guamanian amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia (PD), typical NFT appear in large motor neurons which contain a high, up-regulated mRNA for amyloid β -protein. We have also studied regulation of mRNA and precursor protein expression in hippocampal neurons and in endothelial cells *in vitro*. Thus, continued molecular and cell biology studies of brain amyloid β -protein biosynthesis, processing and regulation will surely continue to dominate research on AD and aging brain for some time.

The discovery that the subunit of aging brain amyloid in AD and Down's syndrome had no amino acid homology with the much larger monomer of scrapie-kuru-CJD amyloid in scrapie-associated fibrils (SAFs) or kuru plaques led to the clear differentiation of transmissible from nontransmissible brain amyloidoses. Scrapie and the transmissible dementias (kuru, CJD and its GSS variant) form the amyloid of SAF and scrapie or kuru plaques from a proteolytically cleaved portion of the larger infectious form of the scrapie amyloid precursor protein. The genes for aging brain amyloid precursor and for the scrapie amyloid precursor show no sequence homology and the amyloidogenic subunits cleaved from the full-length precursors are extremely different. The subunit protein for the nontransmissible brain amyloidoses of aging and AD is a polypeptide of 4.1 kDa (42 amino acids) in size and is nonglycosylated while that from scrapie-kuru-CJD is 27 kDa in size and has two glycosylated sites. In humans, the genes for the aging brain amyloid precursor and for the scrapie amyloid precursor are on chromosomes 21 and 20, respectively.

We have established a multifaceted research program to investigate the pathogenetic mechanisms underlying the brain amyloidoses. These studies include *in vitro* characterization of amyloid fibrils formation from synthetic polypeptides. Using the unique resource of our tissue bank of specimens from experimental animals, we have initiated studies on the structure of amyloid protein in different strains of virus from the same host. Virus properties of pathogenesis, incubation time, distribution of lesions and host range must depend on secondary and tertiary configurational changes in a host precursor when no amino acid sequence pleomorphism can be shown between virus strains from a single breed of host.

Our multidisciplinary approach to the study of high-incidence motor neuron disease, conducted during the past three decades among geographically and genetically diverse groups in the western Pacific, indicates unequivocally that there is no genetic cause, but rather a defect in mineral metabolism, provoked by chronic nutritional deficiencies of calcium, which leads to increased intestinal absorption of toxic metals and the codeposition of calcium, aluminum and silicon as aluminosilicates and calcium hydroxyapatites in affected neurons. This elemental deposition interferes with slow axonal transport by altering neurofilament biosynthesis and/or catabolism, resulting in excessive neurofilament accumulation in motor neurons, the ultrastructural hallmark of ALS. At present, the greatly increased life expectancy of Guamanians has produced the problem of differentiating the expected cases of AD in aged Guamanians from the previously more clear-cut cases of PD in younger subjects, now no longer seen.

Studies in nonhuman primates to develop an experimental animal model for AIDS encephalopathy and to evaluate humoral responses induced by potential vaccines against HIV infection continue to occupy a significant portion of our resources. In addition, attempts are underway to detect novel retroviruses in chimpanzees who may have CD4+ lymphocytopenia.

In collaboration with Prof. Pamela Rodgers-Johnson, who is investigating the epidemiology of schizophrenia in Jamaica, 202 sera from schizophrenics were screened for evidence of exposure to HIV-1, HIV-2, HTLV-I and HTLV-II. (It was found that 3 (1.4%) sera were Western blot positive for HIV-1 and 20 (9.9%) were positive for HTLV-I.) There were no HIV-2 or HTLV-II seropositives detected. This prevalence rate does not differ significantly from that found in adult Jamaicans.

Our work on the hantaviruses, causative agents of hemorrhagic fever with renal syndrome (HFRS), continues as a worldwide collaborative effort. Our effort has focused on elucidating the epidemiologic, epizootologic and virologic aspects of HFRS in Yugoslavia, where we have isolated a new serotype of hantavirus (Belgrade virus) causing the severe form of HFRS. Still other hantavirus strains, isolated from mice and voles captured in HFRS-endemic regions in Yugoslavia, are currently being characterized.

We are continuing our studies on the mechanism of language acquisition, including the naturalistic observation of extreme polylinguality. We are organizing a Fondation pour l'Etude du Systeme Nerveux Workshop in Geneva in April 1993 on The Origin, Evolution and Neurobiology of Language. Our comparative inquiries on widely divergent styles of psychosexual development in children from diverse cultural milieus continue to yield new data on neurologic programming which departs from "normal" behavior much further than previously imagined by most psychiatrists, psychologists, sociologists, and anthropologists. The gene determining the male pseudohermaphroditism in the Simbari Anga people has been isolated and is being further studied in genetic epidemiologic studies of this affected population.

We have always studied not only the clinical and laboratory aspects of neurologic syndromes, but also the social and public health implications of these syndromes. Our long-term data gathering of socio-epidemiological observations concerning kuru and pseudohermaphroditism in Papua New Guinea, cysticercosis epilepsy in West New Guinea, chronic goitrous cretinism in West New Guinea, ALS and PD in Guam and West New Guinea, and Viliuisk encephalomyelitis and Pierre-Marie olivopontocerebellar degeneration in the Sakha Republic, as well as other neurodegenerative disorders, continue to provide valuable insights on cultural reactions to on going epidemic and endemic diseases and to suggest alternative social responses to such catastrophes. Much that we learn is applicable, by extrapolation, to contemporary

problems we face in the United States, such as AD and senile dementia, the AIDS epidemic, and the seemingly uncontrollable illicit use of drugs.

Honors

During the past year Richard Yanagihara of our laboratory has won the Bailey K. Ashford Medal of the American Society of Tropical Medicine and Hygiene for his work on hantavirus infections and the discovery of new quasi-species of proto-Melanesian HTLV-I. The Laboratory Chief received the major Award and Medal of the Third International Conference on Alzheimer's Disease and Related Disorders in Abano Terme, Padova, Italy for "his pioneering studies on the transmissible dementias and relationship of these diseases to Alzheimer's disease"; was elected a Fellow of the Royal College of Physicians (Edinburgh), was named the Rubbo Orator by the Australian Society of Microbiology, and was awarded the Laurea Honoris Causa in Veterinary Medicine by the University of Milan.

Recent alumni of the laboratory have also received major honors: Pamela Rodgers-Johnson, University of West Indies (elected to the Third World Academy of Sciences); Colin Masters, University of Melbourne (Potemkin Award, Alzheimer's Disease Society); Dmitry Goldgaber, SUNY at Stonybrook (Metropolitan Life Award for Research on Alzheimer's Disease and Aging); Michael Alpers, Papua New Guinea Institute of Medical Research (McDonald Prize, Royal Society of Tropical Medicine and Hygiene and elected to the Third World Academy of Sciences); Roger Traub, IBM Thomas Watson Research Center (Research Award in Basic Neuroscience, American Epilepsy Society).

Slow Unconventional Viruses Causing Transmissible Brain Amyloidoses

Our laboratory has concentrated on clarifying the relationship between the viruses of kuru, CJD, and scrapie and their host-specified precursor proteins. We now know that scrapie virus is the monomeric form of the configurationally changed 35-37 kDa precursor protein present in normal brain and in infected brain tissues, but modified by infection to a less soluble protease-resistant form which is infectious. The 27-30 kDa scrapie amyloid protein (prion protein; PrP²⁷⁻³⁰), which assembles *in vitro* into Congoophilic, birefringent rods resembling the scrapie-associated fibrils (SAF) of Merz and into kuru-CJD-scrapie plaques is also infectious, even as a monomer. We have now demonstrated that this normal host protein modified by scrapie virus infection is itself the infectious agent (an amyloid molecule, autoinducing the modification of host protein precursor into its infectious form). No nonhost nucleic acid is present, even in highly infectious preparations. The improbable conjecture that the entire infectious process is that of an autonucleated and autopatterned conformational change of a protein precursor which leads to crystallization and polymerization forming amyloid fibers of SAF and kuru plaques is now verified. The host gene specifying the 35-37 kDa precursor protein, which is on chromosome 20 in man and 2 in mice, has been fully sequenced.

Our continuing study of the scrapie amyloid protein indicates that this protein is formed by N-terminal cleavage of the infectious isoform of the scrapie precursor and that the polymeric forms are highly infectious. Even when purified to homogeneity, the dissociated monomers retain infectivity. The detailed compositional analysis and physicochemical behavior of the monomer demonstrated no additional macromolecular-forming scrapie infectious unit. Thus, the scrapie amyloid protein and also its full-length, polymeric or monomeric precursor are infectious. The precursor has two complex branched sugar chains with an unusually high fucose and sialic acid content. However, they do not contribute significantly to the infectivity; are not essential for scrapie amyloid polymerization; and are located on the external surface of the amyloid fibrils.

During scrapie and CJD infection, the normal precursor is converted into a protease-resistant infectious form. Its glycolipid anchor resembles other anchoring glycolipids. Moreover, chemical cleavage of the polysaccharides does not alter infectivity. Thus, the results do not indicate a structurally different glycolipid but rather different topology and conformation of the infectious form in the membranes and suggest a pattern-inducing a "replicating" change in the tertiary structure.

Polyclonal and monoclonal antibodies prepared against synthetic polypeptides of the N-terminus of the scrapie amyloid reveal varying distribution and patterns of the epitopes in normal and infected tissues. Such antibodies have shown reactivity to the scrapie-associated proteins and, to our surprise, to many purified proteins, including purified natural and synthetic human growth hormone. However, these antibodies to SAFs or to the synthetic polypeptide specifically label purified SAFs from kuru-, CJD- and scrapie-infected brains. Such SAFs are not obtainable from brains of other human neurodegenerative diseases.

These immunocytochemical and molecular biologic studies on the scrapie/kuru/CJD-associated proteins and their normal precursors are largely aimed at preparing them in high purity and sufficient amounts for crystallographic study, and investigating at the organic chemical level, the fine structural modification involved in the conversion of normal host-protein into amyloid fibers which appears to be the major pathogenic reaction of these diseases.

Genetic Control of De Novo Generation of Infectious Amyloid Protein from Host Precursors

For more than two decades we have carefully preserved, at temperatures below -70°C, tissues from chimpanzees and other nonhuman primates affected with the human CJD and GSS viruses, kuru virus, and scrapie virus, and tissues collected from cats, hamsters, guinea pigs and other animals susceptible to these viruses. These were collected and saved for eventual biochemical study when this would be possible. Newer polymerase chain reaction (PCR) techniques on long-stored tissues from these collections and from crucial cases of familial CJD and GSS have yielded critical data on the several point mutations underlying the spontaneous generation of infectious amyloid proteins from host precursors in CJD and GSS. This has led to the association of this mutation with CJD in Eastern-European Slavs and in circum-Mediterranean Sephardic Jews from Tunis, Greece, and Israel. It is now possible to process these tissues for PrP 27-30 protein, SAFs, and the 33-35 kDa scrapie-specific protein and its precursor. As more sophisticated study of the structure of these proteins becomes possible, we hope to determine from this material the contribution of the host to these subacute spongiform encephalopathy viruses or slow unconventional viruses. This we are in a unique position to do, since it would take from two years to over a decade for other laboratories to obtain infected brain material from a number of different species, each inoculated with the same strain of virus.

We have indications that there are many strains of CJD viruses. Using these tissues, it is possible to answer the critical question of the relative contributions of the host and the virus strain to the pathogenesis of the disorders and the molecular structure of the virus strains. Since we expect all strains or passage lines of kuru-CJD-scrapie viruses to replicate by an infectious transformation of the normal host precursor protein into an infectious configuration, it follows that all virus strains should produce progeny in a given host which have the identical host amino acid sequence in the infectious monomer. Strain differences determined by the host precursor gene or its mutations would not "breed true," i.e., they would not be carried into the progeny. Thus, since we do find strain differences in viruses from the same host, this would require a different explanation than conservation of genotypic identity. Rather, we should expect a conservation of secondary and tertiary configurational change by autonucleation and autopatterning epitaxial crystal replication and growth. The stored frozen brain passage material, particularly of different viruses (scrapie-kuru-CJD-GSS) passed into the same breed of host, is being used to resolve this critical matter.

The Biochemistry and Immunochemistry of Scrapie Amyloid Protein and Precursors

The continuing study of scrapie amyloid protein has demonstrated that this protein is formed by N-terminal cleavage of the infectious isoform of the scrapie precursor and that the polymeric forms are highly infectious. Even when purified to homogeneity, the dissociated monomers retain infectivity. The detailed compositional analysis and physicochemical behavior of the monomer demonstrated no additional macromolecule-forming scrapie infectious unit. Thus, the scrapie

amyloid protein and also its full-length, polymeric or monomeric precursor, appears to be infectious. The precursor has two complex branched sugar chains with an unusually high fucose and sialic acid content. However, they do not contribute significantly to the infectivity; are not essential for scrapie amyloid polymerization; and are located on the external surface of amyloid fibrils.

During scrapie and CJD infection, the normal precursor is converted into a protease-resistant, infectious form with different membrane interactions and different physicochemical behavior. The glycolipid anchor of the protein is being investigated, but we are currently unable to detect any differences between this and other anchoring glycolipids. Moreover, chemical cleavage did not alter infectivity. Thus, the results do not indicate a structurally different glycolipid but rather different topology and conformation of infectious form in the membranes and suggest a self-replicating change in the tertiary structure.

We have developed and characterized new sets of species-specific polyclonal antibodies against HPLC-purified, full-length PrP protein and different N- and C-terminus segments of the molecule to follow posttranslational processing and amyloid formation during scrapie and CJD infection *in situ* by immunocytochemistry. The highly specific antibodies will increase the sensitivity of the diagnostic test for scrapie and CJD which is also under the development.

The Structure-Function Relationship and In Vitro Scrapie Amyloid Protein Formation

Understanding the transition from normal to infectious precursor protein, tertiary and quaternary structure, its thermodynamics, and stoichiometry is critical to: investigating the spongiform encephalopathies; studying aging brain amyloidosis (A4 amyloid, Alzheimer disease amyloid); and investigating the basic mechanism of the assembly of structured protein monomers into the microscopic and macroscopic range. The work on the structure of the infectious precursor of hamster scrapie amyloid and on the molecular mechanism of assembly into amyloid fibrils by using circular dichroism (CD) spectroscopy and infrared (IR) spectroscopy is in progress.

In familial cases of the spongiform encephalopathies, the transition from PrP^C to PrP^{CJD} is apparently spontaneous, with the highest probability in GSS families. However, this hypothesis must be validated *in vitro*, and furthermore, we need to understand the molecular mechanism of the transition at the level of secondary and tertiary structure and/or possible ligands and their relationship to infectivity. Using the baculovirus expression vector system, we have expressed wild-type human prion protein gene (PRIP gene) and two mutations, one at codon 102 (Pro to Leu), linked with development of GSS and the other at codon 200, linked with development of familial CJD. We observed no difference in protease cleavage kinetics, physicochemical behavior, and glycosylation between the wild-type recombinant PrP or those carrying mutations in codons 102 or 200. The apparent Mr, physicochemical behavior, high hydrophobicity, and negative results of glycolipid cleavage suggest that all human recombinant PrP proteins maintain a C-terminal hydrophobic domain. In confirmation, the proteins constructed with no C-terminus domain were secreted from Hi5 cells.

The access to secreted PrP proteins will facilitate the structural studies on PrP by CD spectroscopy. The long-term bioassay study on experimental animals is in its second year.

Bovine Spongiform Encephalopathy and Experimental Scrapie in Cattle

The occurrence of bovine spongiform encephalopathy (BSE) in Great Britain (mad cow disease) has raised several major problems, the exact route of transmission to cattle, and the possible risk to man. Understanding of the mechanism and the possibility of vertical transmission to offspring, and possible spread of scrapie into new species across the species barrier is necessary for the development rational preventive measures. We are continuing the study of wild strains of Angora

goat and sheep scrapie inoculated into cows and reisolated in LacJ and NIH inbred mice and hamsters as a model for the possible change in biologic properties after passage through cattle.

Moreover, brain tissue from British BSE brains tested positive for scrapie amyloid protein by conventional Diringer isolation technique followed by Western blot and also by alternative dot blot procedure, and has caused spongiform encephalopathy in several susceptible rodent species.

No means of investigating host range and virulence from the genomic sequence of the scrapie amyloid precursor is yet available. We are trying to approach this problem.

We have developed technique for decontamination scrapie and BSE- infected brains. Experiments eliminating scrapie from preparations of gangliosides have important implications in the development of safe biologicals where cows are the primary and only the source.

Nontransmissible Brain Amyloidoses of Aging, Alzheimer's Disease (AD) and Other Dementias

Amino acid sequencing of the 4 kDa polypeptide subunit of the paired helical filaments (PHF) of neurofibrillary tangles (NFTs), amyloid plaque cores, and amorphous amyloid in Congophilic angiopathy indicates that all three pathognomonic structures of the aging brain, AD, Pick's disease, progressive supranuclear palsy (PSP), late Down's syndrome, Guamanian ALS and PD and von Economo's encephalitis are composed of identical 4 kDa (42 amino acids) subunits. In the preparations of purified PHF from NFTs of Guamanian ALS and PD, no extracellular amyloid in the form of amyloid plaques or vascular amyloid deposits were present to produce possible contamination. This 4 kDa polypeptide subunit (β -amyloid protein) which easily associates into dimers, tetramers, octamers, and hexadecamers, shows no amino acid sequence homology to the infectious scrapie amyloid subunit of the transmissible cerebral amyloidoses. Our recent studies indicate that β -amyloid protein is also the constituent of the NFTs found in neurologically normal Guamanians with neurofibrillary degeneration.

Genetically determined familial AD is caused by a mutation in a different gene, not that for the β -amyloid protein precursor, which must determine a molecule involved in the rapid turnover of this protein. In the Dutch families with autosomal dominant hereditary cerebral hemorrhage, with amyloidotic angiopathy, however, a point mutation in base 1852 produces a replacement at amino acid 618 of glutamic acid by glutamine, and this is amino acid 22 in the amyloid subunit.

Neurofibrillary Pathology in Human Neurodegenerative Disease

The gene encoding the amyloid β -protein has been shown to be highly conserved in evolution and is expressed in various human and animal tissues. As a complex transcriptional unit, it utilizes alternative splicing; alternative spliced forms of the amyloid β -protein precursor cDNAs contain 50% homology to the Kunitz family of serine protease inhibitors. It may be the absence, inhibition or overexpression of these alternative forms that modify the host precursor proteins leading to production of amyloid β -protein.

These modified forms of the amyloid β -protein in its microfibril or oligomeric forms, like the fibril amyloid enhancing factor (FAEF) in AA amyloidoses, may act as amyloid-enhancing factors, or as nucleants or niduses that accelerate its own formation by self-polymerization and copolymerization with other molecules like glycosaminoglycans (GAGs) leading to amyloid deposition. We have demonstrated these GAGs in amyloid plaques and NFTs in AD, normal aging brain, Down's syndrome and Guamanian ALS and PD. In AD and Guamanian PD, the 42-amino acid subunits of the β -amyloid of NFTs, amyloid plaque core, and Congophilic angiopathy could themselves serve, in the form of oligomers or fibril microfragments, as nuclei that enhance their deposition as amyloid.

Previously, we found that the conditions under which neuronal synthesis of amyloid β -protein might contribute to the formation of NFTs was through amyloid β -protein precursor (APP) mRNA

expression in developing fetal rabbit and mouse hippocampal neurons *in vitro*. Currently, studies are underway to specifically determine the expression of APP mRNA isoforms 695 and 751 during early neuronal development *in vitro*. These studies should greatly enhance our understanding of the formation of β -amyloid in the brain and complement the recent studies of its deposition in transgenic mice. Likewise, studies are underway using primary suspensions of NFTs (β -amyloid) from the hippocampus of Guamanian patients with ALS and PD to initiate the process of neuronal degeneration and NFT formation in nonhuman primates and rabbits.

New neuropathologic observations on Pacific ALS and PD (Guam and Kii Peninsula foci) led to our discovery of rare neuropil threads composed of microtubule-associated protein tau (MAP-tau) in NFTs in addition to the β A4 amyloid found earlier. We confirmed the absence of vascular amyloid and senile plaques in the hippocampus and adjacent entorhinal cortex of those younger ALS and PD patients. These observations indicate that although there is a strong neuropathologic link between Pacific ALS and PD and AD, there may be some important differences in the specific pathogenic pathways that are responsible for the deposition and altered distribution of MAP-tau and intra- and extracellular β A4 amyloid. Other recent studies on ALS, PD and AD from our laboratory have demonstrated that lipofuscin-enriched neurons were found in the hippocampus in a distribution (Ca4>Ca3>CA2>CA1>subiculum) that was exactly opposite to that of NFTs. These observations suggest that the process of lipofuscinosis leading to the noncytotoxic inert endproduct lipofuscin in neurons protects against amyloid fiber polymerization necessary for NFT formation.

Toward a Biochemistry of Silicon and Aluminum

The metabolic adjustment to severe environmental deficiency of calcium and magnesium which is responsible for the deposition of calcium, aluminum, silicon, phosphorous and other minerals in brain cells in early life in the high-incidence foci of ALS and PD and the early appearance of Alzheimer's NFTs in isolated populations in the western Pacific (Guam, Japan, West New Guinea) was first suggested by epidemiologic and ecologic studies. Mineral analyses of environmental specimens of soil and water confirmed this hypotheses. Finally, electron-probe X-ray microanalyses, using both energy- and wavelength-dispersive spectrometry, and secondary ion mass spectrometry have demonstrated these long-term deposits in NFT-bearing hippocampal neurons of Guamanian ALS and PD patients and of normal individuals exposed to the same environmental deficiencies. When these calcium and magnesium deficiencies are removed by increased access to outside foodstuffs, changed water supply, and improved transportation and economy, all three diseases (AD, ALS and PD) have declined markedly in incidence or disappeared within a period of two or three decades. This discovery of the primary cause of all three pathologic processes in the western Pacific isolates has led to animal experiments which further substantiate the hypotheses and stimulated a renewed interest in the role of mineral deposition in interfering with axonal transport. Even therapeutic and prophylactic clinical regimens are now suggested and some are under study, including the chelation of aluminum by desferioxamine in an AD clinical trial which showed a 50% improvement in the patients.

Furthermore, the role of silicon and its polymers in altering the secondary structure of proteins through long series of hydrogen bonds is now under investigation. Silicon and aluminum compounds can interact strongly with phospholipids, lipids, carbohydrates and oligonucleotides as well as with polypeptides. Thus, mineral deposits of montmorillonite clays—calcium-aluminum-silicates—and hydroxyapatites can denature and alter protein fine structure and conceivably play an active role in degradation of host precursor proteins to amyloids.

The recent confirmation of older observations of silicon-containing deposits in the center of purified insoluble amyloid plaque cores from AD patients and in AD NFTs has greatly stimulated interest in the possible role of these silicon- and aluminum-containing mineral deposits as nucleating agents or even as autocatalytic agents in the deposition or crystallization of such amyloid deposits. Recent research on the involvement of aluminum in the pathogenic process of AD has been and continues to be presented at many international meetings by a growing cadre of scientists who no longer view aluminum as a "harmless" agent. The emphasis has clearly shifted from purely descriptive associations between aluminum and other elements and human disease to a much more mechanistic and quantitative approach to understanding how aluminum is involved in the disease process.

In Vivo and In Vitro Models of Motor Neuron Degeneration and Pathogenesis

We have produced several new provocative experimental animal models of motor neuron disease and neurofibrillary degeneration, using aluminum salts. These models demonstrate that chronic low-dose intracisternal inoculations of New Zealand white rabbits leads to a spastic myelopathy characterized by hyperreflexia, hypertonia, gait disturbances, muscle weakness, and neurogenic atrophy. Neuropathologically, this model exhibits extensive topographically specific neurofilamentous inclusions in motor neurons and select brainstem nuclei. This past year we have also determined that the intracisternal inoculation of aluminum phosphate leads to reduced MAP-tau in motor neuron dendrites and a reduced number of dendritic trees, both of which have been confirmed at the ultrastructural level. These observations suggest that dendritic degeneration is characteristically involved in aluminum intoxication in addition to the abnormal accumulation of neurofilament protein in perikarya and axons, demonstrating clearly the utility of the chronic low-dose aluminum model for the study of neurofibrillary degeneration and the intraneuronal pooling of cytoskeletal proteins.

We have also developed a chronic model of neurofibrillary degeneration using dissociated fetal mouse and rabbit motor neurons in cell culture in order to demonstrate the mechanisms by which aluminum intoxication proceeds. We have clearly demonstrated that micromolar concentrations of aluminum produce neuron-specific thresholds of toxicity leading to intracytoplasmic and intraaxonal pathologies similar to that in human ALS. Utilizing these cultures, ongoing studies are mapping the coexpression of neurofilaments and neuronal enzymes during normal neuronal maturation *in vitro*. Employing immunohistochemical techniques, these neuronal components are identified *in situ* and with sensitive neurobiologic assays, and their syntheses are being quantified. Subsequent studies will explore the expression of these elements under aberrant environmental conditions.

Finally, we have discovered a new neurotoxin, N-butylbenzene sulfonamide (NBBS), a plasticizer used commercially in the polymerization of polyamide compounds. This compound appears to be a true slow-acting organotoxin, one of the few of its kind, that induces a spastic myelopathy in rabbits, accompanied by neuroaxonal degeneration and postsynaptic changes, and raises questions about the safety of NBBS for humans. Studies are underway to develop *in vitro* assays using primary motor neuron and hippocampal neuron cultures, and a sensitive NBBS assay to evaluate the kinetics of this neurotoxin in rats, rabbits and nonhuman primates.

The above studies are a direct outgrowth of our research on high-incidence foci of motor neuron disease in the western Pacific which has resulted in a long-term program aimed at identifying the cellular and molecular mechanisms of aluminum intoxication. It is clear that the development of these *in vivo* and *in vitro* models of low-dose aluminum toxicity hold great promise for providing a better understanding of the pathogenetic processes involved in neurodegenerative disorders, such as motor neuron disease, parkinsonism and AD.

Natural Experimental Models of Aluminum Intoxication in Fish from Acid-Rain Lakes

Abnormal accumulations of cytoskeletal proteins are hallmark characteristics of ALS, PD, and AD. As a direct outgrowth of our long-term systematic studies of ALS and PD in the Western Pacific that have strongly implicated aluminum as a causal factor in the disease process, we are investigating natural models of aluminum toxicity in fish from acid-rain lakes. During the past two years in the Adirondack Mountains of New York, we have studied fish from acid-rain lakes in order to improve our understanding about the cellular and molecular mechanism of aluminum intoxication and the bioavailability of aluminum. It is well known that fish, exposed acutely and chronically to waters acidified by acid rain in regions with low calcium, die of aluminum intoxication, with the primary target organ in mature fish being the gills, where aluminum interferes with ionic- and osmoregulation. However, such a natural model has not been exploited for what it can tell us about the chronic effects of aluminum on the nervous and skeletal systems, the mechanism of aluminum intoxication and the identification of bioactive forms of aluminum (e.g., mononuclear and polynuclear complexes). In

collaboration with wildlife biologists from the New York State Department of Environmental Conservation, we have examined a number of species of fish from acid-rain lakes. Initial and preliminary results suggest that the CNS of these animals has neuropathologic changes, such as chromatolysis, perikaryal and neuritic inclusions and plaque-like structures consisting of aggregated cytoskeletal proteins including neurofilament, MAP-tau, and ubiquitin. There are also indications of neuronal loss in the forebrain of some fish. Aluminum accumulation occurs in the gills of most fish from the aluminum-rich lakes, and aluminum has been found to be deposited in the olfactory rosette and olfactory bulb in the CNS of these fish. This represents a direct intoxication route to the nervous system and we already know that in ALS, PD and AD, the olfactory neuronal pathway is impaired, as it is in rabbits experimentally intoxicated with aluminum. In addition, we have preliminary evidence to indicate a significant loss of neurons in the olfactory region of such fish. These studies, while still in their infancy are very provocative and hold great promise for helping us understand how aluminum alters the nervous system, what forms of aluminum are toxic and how they may play a role in the pathogenesis of human neurodegenerative disorders of unknown etiology characterized by late-onset and slow progression.

Human Lentivirus (AIDS) Encephalomyelitis in Children

Nearly all cases of childhood AIDS, acquired congenitally from a human immunodeficiency virus (HIV)-infected mother, develop a primary encephalitis characterized by dysarthria with eventual aphasia, severe midline truncal ataxia and loss of developmental milestones. The precise mechanisms by which infants of HIV-infected women become infected with HIV are unknown. Estimates of the vertical transmission rate range from 22-80%, with most studies suggesting a risk of approximately 30%. Several clinical, immunologic, and serologic arguments support the hypothesis of transplacental transmission of HIV. Transplacental infection can occur by passage of either cell-free or cell-associated virus into the fetal compartment, leading to infection, or by direct infection of the placenta itself.

We have obtained specimens (maternal blood, placenta tissue and cord blood) from HIV antibody-positive women and their children at the time of delivery. Maternal and cord lymphocytes have been stimulated with PHA, and IL-2 has been added to the blood cultures. Cocultivation has been performed with stimulated blood donor cells. The placenta culture supernatant has been inoculated into stimulated donor peripheral blood lymphocytes.

These ongoing studies will examine the titer of HIV-2 in the serum and CD4-positive cells of the mother at the time of delivery, in the placenta, and in the serum and CD4-positive cells of the cord blood of the child to determine if the virus load of the mother is correlated with infection of the placenta and the child.

The human lentivirus has thus become the major cause of encephalitic death among children and adults in the United States. In adults and more frequently in children, primary encephalitis from human lentivirus infection may occur without an immune deficiency syndrome. Thus, we are dealing with another example of transmissible virus infection resulting in a chronic dementia.

Use of HIV- and HTLV-I-Infected Chimpanzees for Studies of Virus Evolution and Vaccine Development

Important insights have been gained into the pathogenesis and virology of HIV and HTLV-I infection from our experimental infection of nonhuman primates, particularly chimpanzees. Using the earliest HIV-infected chimpanzees, we demonstrated the hypervariable loop of the HIV major envelope glycoprotein to be a dominant neutralizing epitope, clearly indicating the problem this would cause for conventional schemes of immunization. These chronically HIV-infected chimpanzees have proven valuable in the further delineation of antigenic drift of HIV. The hypervariability of the V3 loop and the antigenic drift have been later confirmed in HIV-infected humans. As part of the safety testing of the gp120-depleted killed HIV immunogen, four chimpanzees are being monitored serologically, virologically and molecularly by PCR. Antibodies have been persistently elevated in these animals but detection of the p24 antigen in the media of cultured lymphocytes has been sporadic.

Much has also been learned about HTLV-I infection in humans by studying chimpanzees persistently infected with HTLV-I, as well as HTLV-I-related viruses found in chimpanzees. For example, nucleotide sequence analysis of the HTLV-I pol gene in chimpanzees inoculated six years earlier with HTLV-I indicates a high degree of sequence conservation. These HTLV-I-infected chimpanzees will soon be challenged with Melanesian variants of HTLV-I to determine whether the *in vitro* cross-neutralization observed between cosmopolitan and Melanesian strains of HTLV-I is operative *in vivo*.

Studies are continuing on chimpanzees chronically infected with HIV in the last year, HIV P24 was detected in cultured lymphocytes from 11 or 19 antibody positive chimpanzees which were inoculated between 1983 and 1985. Thirteen of the 19 contained HIV proviral DNA when tested by PCR. Antibody levels remained high in the animals regardless of whether HIV antigen or DNA were detectable, suggesting that even the antigen or DNA negative animals were, in fact, infected. None of the chimpanzees have shown evidence of T-cell depletion or have developed any illness associated with immunosuppression.

Sequencing of the pol-gag region of HIV recovered from frozen lymphocytes of October, 1984 and March, 1992, from a chimpanzee inoculated with HTLV-IIIB in May, 1984, showed that few changes had occurred over the 8-year period. However, sequences from both of these samples differed significantly from the inoculum, prototype HTLV-IIIB. Studies are underway to examine changes in the variable regions of the envelope.

In 1989, we confirmed hairy cell leukemia in a rhesus macaque which had antibodies against HTLV-I or a related agent and had developed meningioma. Leucocyte cultures from that animal produced adherent megakaryocyte-like cells that are still slowly replicating three years later. When fluid from his culture was added to IL-2 stimulated normal human lymphocytes, up to 3% of the human lymphocytes became positive for hairy cell marker within two weeks, as determined by flow cytometry. Further studies are underway using graded filters to determine the size of this factor.

We continued to monitor four chimpanzees comprising the GPL-120 depleted killed HIV immunogen safety testing investigation. The chimpanzees were tested 6 times for virologic and serologic evidence of HIV infection, and twice examined by PCR in the last year. P-24 antigen was detected on 3 of 6 attempts from the control chimpanzee (A189A) which had not received the immunogen prior to challenge. Chimpanzee A3, which had tested negative for P-24 antigen since receiving the immunogen, tested positive in July, 1991, but no antigen could be recovered from 5 subsequent samples through April, 1992. This animal exhibited a strong signal against HIV proviral DNA on samples from September and December, 1991. Antibody levels remained stable throughout the year for each animal.

Search for an Animal Model of AIDS

Our laboratory was first to demonstrate active infection of chimpanzees with human immunodeficiency virus (HIV) (formerly LAV and HTLV-III) and with primary human blood, brain and visceral tissues obtained from AIDS patients. Such chimpanzees developing primary infection after inoculation with human brain tissue from AIDS patients provided the first demonstration of the live virus in the brain of AIDS patients. Since such infection has occurred, even at high dilutions of suspensions of brain tissues from AIDS patients, the presumption is that the virus is present in brain and in high titer. The animals develop virus-specific antibodies but no clinical disease, and if there is any alteration in immune function, it is a transient lymphocytosis with moderate impairment of lymphocyte function, but no T lymphocyte depletion or a helper-suppressor ratio change equivalent to that in human AIDS. Nineteen chimpanzees remain HIV antibody-positive 7 to 9 years postinoculation, and of these, 13 have detectable HIV genomic sequences in their lymphocytes by PCR. However, as evidenced by the inconsistency with which HIV can be detected by virus isolation or gene amplification, the viral copy number in peripheral blood mononuclear cells must be extraordinarily low.

Many other species of nonhuman primates have been inoculated without producing disease, primary infection, or antibody conversion; however, an occasional rhesus monkey inoculated with these human viruses has seroconverted. The human lentiviruses do not produce disease in nonhuman primates, even though they are very closely related to simian immunodeficiency virus. Thus, we are without a good experimental model for vaccine evaluation in small animals or in nonhuman primates, and all that can be done at present is to test for the ability of vaccines to protect against primary infection.

Search for Animal Model of HTLV-I

We have reported a model in severe combined immunodeficiency mice (SCID) inoculated with HTLV-I cells derived from patients with HAM/TSP. C.B-17 SCID mice were injected with twenty million 87-390 (Jamaican), 87-394 (Colombian), SI.1 and SI.5 (Solomon Islanders) HTLV-I cells. Two days to over six week old mice were used in the studies. Only mice injected with 87-390 cells developed tumors at or near inoculation sites. Cells from intraperitoneal cavity disseminated into hematopoietic, non-hematopoietic organs and in some mice into nervous system. All mice died in less than one hundred days following inoculation. Tumor cells were cultured *in vitro* and were analyzed by flow cytometry. Monoclonal antibodies recognized human T-cell markers on cells recovered from SCID mice tumors. T-Cells recovered from SCID mice were subjected to polymerase chain reaction (PCR), indirect immunofluorescent antibody staining (IFA), and ultrastructural studies of the spinal cord. Sections of tumors and brain tissues were fixed in formalin, stained and examined histologically. Cells stained by IFA were positive for HTLV-I antigens. HTLV-I proviral DNA was detected from tumor cells by the PCR technique. Hind leg paralysis, uveitis and keratoconjunctivitis sicca were observed in some mice. Ultrastructural studies of central nervous system revealed astrocytosis, gliosis, demyelination and infiltration of mononuclear cells. Tumor cells recovered from SCID mice inoculated into baby and adult SCID mice resulted in fatality. In culture, mixture of SCID mice cells and tumor cells (87-390) bearing HTLV-I induced syncytium formation. Angiogenic activity was observed grossly and histologically in newly formed metastasis. Joint swelling was noticed in some mice. The remaining three HTLV-I infected cell lines (87-394, SI.1 and SI.5) inoculated into SCID mice did not induce tumors. Failure of these cells to grow might be due to clearance function of NK cells and macrophages in SCID mice. A HTLV-I successful human (87-390) to mouse xenograft model in this context requires further study. Our preliminary results suggest that SCID mice serves as a potential model for understanding HTLV-I diseases and potential usefulness for therapy of HTLV-I diseases. Further studies are under progress.

Human T Lymphotropic Virus Type II (HTLV-II) Infection Among Amerindians of South America and Indigenous Peoples of Far Eastern Russia

During the past decade, since the discovery of HTLV-I, high prevalences of infection have been found in the Caribbean basin, parts of Central and South America, western and central Africa, southwestern Japan and widely separated regions in Melanesia. Much less is known about the epidemiology and disease potential of HTLV-II, despite being discovered just two years after HTLV-I. Recently, unexpectedly high prevalences of HTLV-II infection have been found among the Navajo of New Mexico, the Seminole/Miccosukee of Florida, the Guaymí of Panama, the isolated Cayapo and Kraho of Brazil, and possibly the Inga and Embera-Waunana of Colombia. To determine whether similarly high prevalences of HTLV-II infection occur among other Amerindian groups in Colombia, we have tested nearly 1,200 sera from members of 18 culturally distinct Indian populations from widely separated regions in Colombia for antibodies against HTLV-I/II. Co-existence of HTLV-I and HTLV-II infection was found among the Wayuu Indians from the Guajira region, while the Tunebo Indians from the Santander region and the Waunana Indians from the Choco region had high prevalences of HTLV-II and HTLV-I infection, respectively. By contrast, surveys for HIV-1 and HIV-2 infection among these Amerindian populations have shown no evidence for infection.

The finding of naturally occurring HTLV-II infection among the Wayuu, a semi-isolated but relatively acculturated Amerindian group inhabiting the Guajira region along the Caribbean coast of Colombia, parallels that discovered among other native American Indian populations. Whether

similarities in cultural practices of the Wayuu and other Amerindians can account for the high prevalences of HTLV-I and HTLV-II infection are unknown. Studies of HTLV-II-infected members of these Amerindian populations should augment our understanding about the disease potential of HTLV-II. Further detailed studies of populations in which HTLV-I and HTLV-II coexist naturally, and may also provide important insights into the early dissemination, as well as phylogeny, of these human retroviruses. Attempts are underway to isolate and characterize these lymphotropic retroviruses from the Wayuu, Tunebo and Waunana Indians of Colombia. In addition, surveys are in progress to ascertain the endemicity of HTLV-II infection among trans-Siberian peoples who are related to the Amerindian ancestors.

Molecular Epidemiology and Evolution of Human T Lymphotropic Virus Type I (HTLV-I)

We continue to pursue the myriad, ever-expanding clinical forms of CNS involvement with HTLV-I and the issues pertaining to the evolution and early dissemination of HTLV-I. Our laboratory has continued to make significant contributions to the former, and to the latter, we have led the discovery of highly divergent molecular variants of HTLV-I from remote Melanesian populations in Papua New Guinea and the Solomon Islands, in which our earlier seroepidemiologic reports of high prevalences of HTLV-I infection were felt by many experts in the field to be false-positive seroreactivity. Our earlier conjecture that variants of HTLV-I are endemic in the western Pacific region has now been verified by the demonstration of such variants, by virus isolation and gene amplification, among Melanesians of Papua New Guinea and the Solomon Islands and among Aborigines of central and northern Australia. We have now studied 12 strains of HTLV-I from Melanesia. Unlike the so-called cosmopolitan prototypes of HTLV-I from the Americas, the Caribbean, Europe, Africa and Japan which exhibit $\geq 97\%$ sequence homology among themselves, these Melanesian variants of HTLV-I diverge by approximately 8% (92% sequence identity) from cosmopolitan strains of HTLV-I. Sequence and phylogenetic analyses of these Melanesian strains of HTLV-I indicate that they are more similar to HTLV-I strains isolated recently from Aboriginal groups from northern and central Australia. The discovery of these highly divergent sequence variants of HTLV-I in isolated Melanesian populations, who have had no contact with Africans, Europeans, Asians and nonhuman primates, suggests that HTLV-I originated somewhere in the Indo-Malay region, rather than in Africa, and evolved independently over many millennia. Although active interspecies transmission of retroviruses probably occurred between humans and nonhuman primates indigenous to Africa and Southeast Asia (Sunda Land and Wallacea) in the distant past, such interaction was not operative in Papua New Guinea, the Solomon Islands and Australia because there have never been nonhuman primates in Melanesia or Australasia.

Unique nucleotide substitutions, recently identified among these HTLV-I variants from Melanesia, have now made possible the direct genotyping of HTLV-I strains by using topotype-specific oligonucleotide-based PCR. By using oligonucleotide primer pairs derived from sequences unique to the gp46- and gp21-encoding regions of the env gene of the Melanesian HTLV-I variants, HTLV-I strains from widely separated geographic regions can be grouped into either of two major geographic-specific genotypes or topotypes: an Austro-Melanesian or paleo-Melanesian topotype and a cosmopolitan topotype. The genetic similarity between the Melanesian and Australian Aboriginal strains of HTLV-I is consistent with the early migration of human populations and the settling of Sahul Land, the then single continent of Papua New Guinea and Australia, more than 30,000 years ago, and with the more recent settling of the islands in Melanesia by Austronesian settlers approximately 5,000 years ago. Thus, the discovery of the paleo-Melanesian variants of HTLV-I has provided important insights into the origin, evolution and global dissemination of this oncogenic lymphotropic retrovirus.

Viliuisk Encephalomyelitis in Sakha (Iakut) People of Northern Siberia

We are preparing a revised third edition of our definitive bibliography on Viliuisk encephalomyelitis (VE), since most reprints are unfamiliar to English-speaking neurologists. Our earlier epidemiologic and clinical studies and a definitive review of Russian investigations of this disease in English has just been published in *Brain*. The disease has continued to spread unabatedly and we are closely assisting our Russian colleagues in the analysis of clinical and epidemiologic data and the laboratory study of postmortem tissues, as well as serum, CSF and leucocytes.

The Laboratory Chief, who has been invited by the Ministry of Health of the Sakha Republic (Iakutia) of the Russian Federation to serve as Scientific Consultant and Honorary Chairman of the Program for the Study of VE. He has, along with two others members of the laboratory, conducted a month-long expedition on VE, returning with hundreds of frozen sera, CSF and leucocytes for studies aimed at establishing the etiology of this chronic brain infection. Intensive laboratory studies are underway, screening for antibodies to viral, rickettsial, chlamydial, bacterial, protozoal, helminthic and fungal antigens, searching for nonhost genomic sequences by PCR techniques and subtractive hybridization, and attempting to isolate *Borrelia* species and mycobacteria. Primates and other laboratory animals inoculated with autopsy tissue and cocultivation of explants from early autopsy tissues with many cell lines are being continued.

VE, recorded among the Iakuts for more than a century, was first reported in 1887 by R.K. Maak, a German ethnologist and explorer. The Iakut people of the Viliui River valley call the disease "bokhoror", a term which means "stiffness" in their Iakut language. The disease has affected Iakut people living in rural regions of the Viliui valley, a population of about 90,000 Iakut people. It does not affect the 70,000 non-Iakut people living among them; Russians have lived in the area for more than two centuries. In the most intensely affected villages of the Viliui valley, over 1% of the population is ill with VE at any one time, and it causes some 5% of the deaths among them.

VE occasionally begins with prodromal symptoms lasting 1 to 3 days, including malaise, insomnia, weakness, apathy, anorexia, abdominal discomfort, nausea, and emesis. More often, VE has an acute onset with high fever (39–40°C) and chills, with an excruciating headache, influenza-like muscle pains, and extreme lethargy. This is accompanied by the sudden appearance of cranial nerve dysfunction, including ophthalmoplegia (ptosis, diplopia, strabismus, impaired convergence and accommodation, and disorders of pupillary reaction), and extrapyramidal rigidity, ataxia, and abnormal deep tendon reflexes. Psychotic disturbances are common: these range from mild dementia and depression to hypochondria and aggressive behavior or delirium. There is usually little meningismus. Frequently there is a history of inordinate exposure to the cold a few days or weeks before the acute onset. The acute phase lasts from a few days to a few months. During this phase CSF shows a low cell count of 5 to 50 leucocytes (mostly mononuclear) per mm³ with a moderately elevated protein. Lueitic serology is negative.

This acute phase is followed, after an interval of full or partial recovery lasting a few weeks to a year or more, by a more insidious progressive panencephalitic syndrome usually with fatal outcome 2 to 5 years after onset. Some patients have a more chronic course and are still alive 10 to 20 years after onset of the chronic progressive phase. Progressive loss of memory and dementia, disturbances of speech, increasing spasticity with a strange characteristic shuffling gait with forward bending, extrapyramidal rigidity, spastic paresis, and signs of cranial nerve involvement prevail in the advanced VE syndrome. However, the clinical course is varied and some patients develop parkinsonism with movement disorders, while others show diencephalic, cerebellar or amyotrophic signs in the chronic progressive stage. A progressive communicating hydrocephalus usually develops, and secondary cerebral atrophy is severe.

Most cases progress to death in 3 to 5 years, while a few patients survive into a state of severe spasticity and global dementia for over 20 years. During the chronic stage the very mild pleocytosis of CSF and elevation of CSF protein may persist or disappear.

In patients who die after a long chronic stage, examination of the brain reveals an internal hydrocephalus in many cases with thickened, often adherent, meninges, and severe cortical atrophy. Demyelination is not usually severe. There is diffuse gliosis particularly involving astrocytes. Tiny foci of inflammation occur throughout the brain with neuronal loss and parenchyma infiltration by mononuclear cells in both white matter and gray matter. There is extensive perivascular cuffing with several layers of mononuclear cells. The foci of neuronal loss range from fresh acute lesions with neuronophagia and active inflammation to old sclerotic focal lesions with intense astroglia.

Arnyloid plaques are often seen, and NFTs have been described. All parts of the brain and cord may be involved.

For a long time VE was recognized only in Viliuisk region, but after 1950, VE cases appeared in neighboring areas along the Viliui River. Of 413 VE cases observed by Petrov from 1951 to 1958, 379 (92%) patients were residents of the Viliuisk region and 34 came from the other raions of the Viliui valley. In recent years, about 70% of the VE patients were diagnosed in the Viliui valley, but about 30% of cases were recorded in the more densely populated central areas of Yakutia, where it was previously unknown. In the Viliuisk raion, the prevalence rate reached 1.8% for males and 2.6% for females in the age group of 40–49 years, and 1.34% of the whole population in the 1960s and 1970s. A similar prevalence seems to have continued through the 1980s and into the 1990s. The higher prevalence among women can be explained because the disease is more rapidly fatal in men. To date, no proved case of VE has been detected outside Yakutia.

The high incidence and mortality of VE in some areas in Yakutia, the further geographic spread, occurrence of the disease among young people with resulting early incapacitation, and absence of any means of prevention or specific therapy contribute to the seriousness of the problem. For more than 35 years, Soviet investigators have made intensive clinical and epidemiologic studies of this unique disease and attempted to determine its cause. Many possibilities were considered. More recent studies of the details of its mode of spread into new, previously unaffected, populations of Yakutia suggest that it is an infectious disease of low communicability, with a pattern of dissemination, long incubation, and latency similar to that of leprosy.

Several infectious agents have been isolated from VE patients, but none of them appear to be etiologically involved. Sarmanova and her colleagues isolated a virus, called Viliuisk virus, in laboratory mice inoculated with CSF, blood, brain, or feces from VE patients. Casals showed that this virus is related to the mouse encephalomyelitis (MEM) GDVII strain and, less closely, to the encephalomyocarditis (EMC) virus. Antibodies against this virus, found in normal rodents, are not found in the sera of VE patients. More recently a putative RNA virus-like agent was isolated from a VE patient and on inoculation in rhesus monkeys caused a low-grade encephalitis. The isolated agent has since been identified as *Acanthamoeba castellanii*, a common laboratory contaminant. The relationship of *A. castellanii* to VE has not been completely resolved, but VE patients do not have antibodies against this free-living amoeba.

There has been one case of apparent VE in a non-Yakut laboratory worker inoculated by needle injection with CSF from a VE patient. This individual developed a typical acute onset after an incubation period of 2 1/2 months, and after a remission of several months, symptoms of progressive subacute VE appeared and led to death in 5 years. Her autopsy showed the pathologic changes of VE. This case substantiates the epidemiologic, clinical and pathologic data indicating that VE is a transmissible infectious disease.

The marked inflammatory response with adherent and thickened meninges, extensive perivascular cuffing, and glial nodules suggests infection with a protozoan parasite, rickettsia, bacterium, fungus or spirochete, more than a virus. Thus, the further pursuit of the etiologic agent is essential for a solution of this problem so serious to the Viliuisk Yakut. The solution will clearly open up a new chapter to our worldwide understanding of chronic infections of the brain.

Search for Cryptic Viral Genomic Sequences in Tissues of Patients with Chronic Neurologic Diseases of Unknown Etiology

Our previous attempts, using conventional serologic and virologic techniques, including inoculation of multiple species of nonhuman primates, to find the causes of chronic neurologic disorders of unknown etiology (such as Kozyevnikov's epilepsy, Rasmussen's chronic encephalitis (epilepsia partialis continua), classical Parkinsonism, multiple sclerosis, amyotrophic lateral sclerosis, schizophrenia and autism in children) have been unsuccessful. Both frozen and fixed tissues from these previously studied cases, some preserved for more than 25 years, have been examined for viral genomic sequences using gene amplification techniques. Oligonucleotide primers specific for multiple structural genes of

RNA viruses (rubella, mumps, measles, picornaviruses, flaviviruses, retroviruses) and DNA viruses (herpes simplex, cytomegalovirus, Epstein-Barr, human herpesvirus 6, varicella-zoster, JC) have been employed using PCR, reverse transcriptase-directed PCR (RT-PCR), and nested PCR.

We examined brain tissues from more than 12 patients with Rasmussen's encephalitis for genomic sequences of the viruses and were unable to confirm data from two groups of investigators who have reported *in situ* hybridization evidence of EBV and CMV infection. We have also failed to find viral sequences for measles, mumps, rubella, and JC viruses in brain tissues from 19 patients with multiple sclerosis by RT-PCR and nested PCR. We were also unable to detect any viral genomic sequences in brain tissues from 63 schizophrenic patients. Similar studies of Viliuisk encephalitis are in progress. Studies using oligonucleotide primers for bacteria and parasites are also in progress. We have successfully used PCR to prepare highly sensitive and specific biotinylated cDNA, and have improved detection and confirmation of the identity of amplified viral and human genomic sequences by hybridization with those probes.

The New Plague: Hantaviruses and Hemorrhagic Fever with Renal Syndrome

We remain the dominant laboratory in America working on hantaviruses. Recently, we have isolated a biologically and genetically distinct hantavirus, designated Belgrade virus, from blood and urine collected from Yugoslavian patients with clinically severe hemorrhagic fever with renal syndrome (HFRS). We have also determined that 2 patients from Bulgaria and 4 patients from Albania with severe HFRS were infected with Belgrade virus, indicating that the geographic distribution of Belgrade virus is not limited to Yugoslavia. The absence of cross-neutralization between Belgrade virus and the 4 known hantavirus serotypes (Hantaan, Seoul, Puumala and Prospect Hill) adds another level of complexity in the development of vaccines for the prevention of HFRS. Sequence analysis of the G2-encoding region of the M segment of Belgrade virus indicates that it differs significantly from Hantaan, Seoul, Puumala and Prospect Hill viruses, but exhibits a close genetic relationship with Dobrava virus, isolated from a yellow-necked field mouse (*Apodemus flavicollis*) captured in Slovenia, suggesting that one of the natural rodent reservoirs of Belgrade virus is *Apodemus flavicollis*. Dendrograms based on these M segment sequences of Belgrade and Dobrava viruses indicate that these hantaviruses form a monophyletic group distinct from Hantaan and Seoul viruses and quite removed from Prospect Hill and Puumala viruses. Our long-held conjecture on the existence of other disease-causing hantaviruses in Yugoslavia and elsewhere in eastern Europe has now been confirmed, and the discovery of Belgrade virus further augments our global concepts about the epidemiology and epizootiology of hantavirus infections.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01282-28 CNSS
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurobiology of Population Isolates Study of Child Growth, Development, Behavior and Learning, and Disease Patterns in Isolated and Primitive Groups		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	D. C. Gajdusek, M.D.	Chief LCNSS
Others:	Clarence J. Gibbs, Jr., Ph.D. David M. Asher, M.D. Paul Brown, M.D. Ralph M. Garruto, Ph.D. Richard Yanagihara, M.D.	Deputy Chief Research Medical Officer Medical Director Supv Research Biologist Medical Director LCNSS LCNSS LCNSS LCNSS LCNSS
COOPERATING UNITS (if any) Continued		
LAB/BRANCH Laboratory of Central Nervous System Studies		
SECTION		
INSTITUTE AND LOCATION NINDS, Bethesda, Maryland 20892		
TOTAL STAFF YEARS-	12	PROFESSIONAL: 8 OTHER: 4
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Studies of human biology of vanishing primitive societies focus on neurologic development and learning patterns in diverse cultural experiments in the human condition found in such isolated groups. Opportunistic investigation of problems phrased by man in isolation is the basis of approach from which most of our studies evolved: <u>kuru-CJD</u>, <u>HIV (AIDS)</u>, <u>HTLV-I</u> slow virus infections of the <u>CNS</u>, <u>aging and Alzheimer's</u>, <u>VE</u>, <u>ALS/PD</u>. Techniques of molecular genetics, biochemistry, immunology, virology, and field epidemiologic, clinical, linguistic and behavioral studies in cultural isolates and genetic and/or geographically isolated primitive bands yield more easily interpretable data than in cosmopolitan societies. Data and specimens from expeditions to Micronesia, Melanesia, Polynesia, South America, Asia and Africa proved valuable in recent <u>HIV (AIDS)</u>, <u>HTLV-I</u>, <u>Hantavirus</u>, <u>JC virus of PML</u> and <u>herpesvirus</u>, <u>CMV</u> and <u>EBV</u> studies. Studies on nutrition, reproduction, fertility, age of puberty and aging, genetic distance and pleomorphisms, unusual and odd higher cortical functions in language learning, cognitive styles, computation (calculation without words or numbers) and culturally modified sexual behavior elucidate alternative forms of neurologic functioning for man which we are unable to investigate once the natural cultural experiments in primitive human isolates are amalgamated into the cosmopolitan community of man. Foci of high incidence of <u>kuru</u>, <u>ALS/PD</u>, <u>HTLV-I</u> myelopathy, <u>epilepsy</u>, <u>familial parkinsonism</u>, <u>Viliuisk encephalopathy</u>, other CNS degenerations, hysterical disorders, schizophrenia, bipolar psychoses, neoplasms, goiter, cretinism, rheumatoid diseases, diabetes, asthma, chronic lung disease, malaria, filariasis, leprosy, cysticercosis, and other infections in these isolated groups have yielded widely significant discoveries. HFRS caused by <u>hantaviruses</u> in Asia, USSR, Europe and newly recognized hantaviruses in the U.S. are studied. Human evolution and adaptability to high altitude, wet or arid climes, variable food supply, mineral deficiencies, toxic exposures and responses to severe diseases or social/psychologic stress are studied in appropriate populations. Thus, <u>HTLV-1</u> and <u>HIV</u> retroviruses as causes of CNS diseases in man were first found in isolated or socially segregated groups: high-incidence TSP focus in Tumaco, Colombia; drug-using mothers in Newark, New Jersey; and are often best studied in these isolated or socially segregated groups. We now have a proto-Melanesian quasispecies of <u>HTLV-I</u> in New Guinea, Solomon Islands, Vanuatu of an archaic origin, not associated with monkeys at least for millenia.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 00969-28 CNSS

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (10 characters or less. Title must fit on one line between the borders.)

Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. C. Gajdusek, M.D.	Chief	LCNSS
Others:	Clarence J. Gibbs, Jr., Ph.D.	Deputy Chief	LCNSS
	David M. Asher, M.D.	Research Medical Officer	LCNSS
	Paul Brown, M.D.	Medical Director	LCNSS
	Ralph M. Garruto, Ph.D.	Supv. Research Biologist	LCNSS
	Richard Yanagihara, M.D.	Medical Director	LCNSS
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COOPERATING UNITS (if any)

Continued

LAB/BRANCH

Laboratory of Central Nervous System Studies

SECTION

INSTITUTE AND LOCATION

NINDS, Bethesda, Maryland 20892

TOTAL STAFF YEARS-	12	PROFESSIONAL:	8	OTHER:	4
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CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input checked="" type="checkbox"/> (a1) Minors		
<input checked="" type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies focus on causes and pathogenesis of chronic degenerative CNS disorders with emphasis on MS; Parkinson's, Pick's, Huntington's and Alzheimer's diseases; ALS/PD of Western Pacific; supranuclear palsy; other presenile dementias; spinocerebellar ataxias; epilepsy; chronic encephalitis with focal epilepsy; Viliuisk encephalopathy; muscular dystrophies; chronic schizophrenia; bipolar psychoses, autism; SSPE; PML; dialysis encephalopathy; goiterous cretinism; cysticercosis; and intracranial neoplasms. We have defined the transmissible and nontransmissible dementias as brain amyloidoses caused by post-translational modification of a specific host precursor protein to amyloid fibril deposits. We now recognize the slow unconventional viruses causing kuru-CJD-scrapie as replicating polypeptides formed *de novo* from a normal host precursor protein, specified on chromosome 20 in man and 2 in mice. The molecular elucidation of the spontaneous configurational change to infectivity, basically a crystallographic problem, is now becoming our major target. Molecular genetic analysis of familial CJD already indicates several point mutations which enormously increase ($\times 10^6$) the probability of this spontaneous *de novo* conversion to an infectious polypeptide. Microbiology must now contend with a totally new paradigm for replicating, infectious, pathogenic agents in the transmissible brain amyloidoses. Our studies focus on the elucidation of the molecular configurational events conferring the property of infectivity on a previously normal host precursor using MRI to elucidate the change in configuration which occurs as transmissibility is produced. In normal aging, Alzheimer's disease (AD), and Down's syndrome a different host precursor protein (specified on chromosome 21 in man, 16 in mice) is a cell-excreted inhibitor of growth factors. Post-translational degradation of this normal precursor forms the 42 amino acid amyloid polypeptide which polymerizes to form the deposits of amyloid angiopathy, amyloid plaques and neurofibrillary tangles in aging, AD and Down's. This occurs in all individuals who reach their 90s. Genetic, toxic, and infectious factors may accelerate this aging brain amyloid deposition.

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CONTRACTS

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New Iberia Research Center
New Iberia, Louisiana

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ANNUAL REPORT

October 1, 1991 - September 30, 1992

Laboratory of Experimental Neuropathology
Basic Neurosciences Program, DIR, NINDS

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ANNUAL REPORT

October 1, 1991 through September 30, 1992

Laboratory of Experimental Neuropathology, DIR

National Institute of Neurological Disorders and Stroke

Henry deF. Webster, Chief

The Laboratory of Experimental Neuropathology (LENP) includes the Cellular Neuropathology Section (CN) and the Neurotoxicology Section (NT). The main goals of the Laboratory's research program are to use molecular and cell biological methods to investigate: 1) glial cell responses and growth factor production during myelin breakdown and regeneration; 2) the pathogenesis and latency of herpes simplex virus (HSV) infections and progressive multifocal leukoencephalopathy (PML); 3) functional and cytotoxic responses of cultured Schwann cells.

The most important new findings in LENP's AIDS research are related to the etiology, pathogenesis, and diagnosis of PML. This fatal viral CNS demyelinating disease caused by JC virus (JCV) is the immediate cause of death in about 5% of AIDS patients, and can represent the sole presenting symptom of clinical AIDS.

1) Etiology: We have shown that 11 JCV coding sequences in PML tissue from the U.S. can be divided into two distinct genotypes. These two DNA-based strain groupings have no apparent serologic consequences, but do have the potential for slightly altering the T-antigen amino acid sequence, and possibly, altering the propensity for quiescent JCV infections to progress to active PML.

2) Pathogenesis: Continued studies in the hamster model of JCV CNS infection have shown JCV expression in endothelial cells of the neonatally infected hamster brain. The immunocytochemical evidence showed greatly enhanced endothelial cell expression in hamsters immunosuppressed with cyclophosphamide. This new finding may yield insights into the pathogenesis of human PML.

3) Diagnosis: Methods were developed for the diagnosis of JCV infection in paraffin-embedded autopsy brain tissues by the polymerase chain reaction (PCR). Thirty-three of 46 cases (72%) studied were positive for JCV DNA sequences by this method. These methods can provide sensitive virus-specific and, eventually, type specific, JCV diagnosis.

Additional observations on AIDS by LENP scientists collaborating with principal investigators in other programs showed that by in situ hybridization, HIV sequences could only be demonstrated in

mononuclear cells and macrophages located in AIDS encephalopathy lesions. In a large series of well-studied cases, sequences were not detected in either neurons or glial cells in sections of brain using optimal processing methods within a few hours of death. These findings provide substantially better evidence than earlier studies which suggested that causes other than direct HIV infection of glia and neurons are responsible for the demyelination and neuronal loss that have been described in HIV encephalopathy. In a project examining mechanisms for bone marrow failure in HIV-infected patients, in situ hybridization and electron microscopic observations showed that replication of several HIV-1 isolates in bone marrow cultures differed as did their inhibition of early erythroid and myeloid colony-forming cells. The inhibition was a relatively late event and was shown to be a phenomenon that was directly related to the virus isolates, that was consistent for each isolate and that was not due to other substances in the culture medium.

Important non AIDS-related LENP discoveries were: 1) Brains from neonates infected with HSV often contain histologic lesions that lack evident viral antigen. We show that HSV DNA sequences can be detected in many such tissue samples using PCR amplification, suggesting a viral etiology for such lesions. 2) HSV-2 (MS strain) infection of lumbar sensory ganglia produced in mice by footpad inoculation resulted in striking neuronal loss. In this and the mouse corneal model, ganglionic infection also led to a selective induction of the neuropeptide galanin, possibly reflecting a regenerative response to neuronal loss. 3) Morphometric, immunocytochemical and in situ hybridization studies of six cases of Baló's concentric sclerosis showed that this rare clinical and pathologic syndrome represents an acute progressive form of multiple sclerosis and that the main changes seen in partially myelinated areas are those found in ongoing myelin breakdown rather than myelin regeneration.

Growth Factors, Glial Cells and Myelin Regeneration

Last year's discovery that cuprizone-induced demyelination induced astrocytic gene expression of insulin-like growth factor-I (IGF-I) was the first in vivo evidence demonstrating that in demyelinating lesions, glial production of growth factors might promote regeneration of myelin. Further study of cuprizone-induced lesions this year included comparisons of relative levels of IGF-I and of glial fibrillary acidic protein (GFAP) mRNAs and peptides produced by astrocytes during the breakdown and regeneration of myelin. Hybridization studies of lesions using specific nucleic acid probes and immunostaining with cell-specific markers showed that levels of GFAP mRNA in hypertrophic astrocytes rose earlier and persisted longer during the recovery period than IGF-I mRNA levels. This suggested that during this pathologic process, astrocytic production of IGF-I and GFAP are independently regulated.

This year, two other models of CNS myelin breakdown and regeneration were selected for new studies of possible growth factor production by reactive glial cells. When focal lesions are produced in the dorsal columns of rat thoracic spinal cords by cryogenic injury, lesion margins contain hypertrophic astrocytes and regenerating, remyelinated axons 30-60 days after injury (Collins and West, 1989). Dr. West produced these cryogenic lesions in groups of rats and preliminary examination in LENP of spinal cords removed and sectioned 7-14 days later showed dramatic increases in astrocytic GFAP mRNA and peptide levels along with levels of IGF-I mRNA significantly above those seen in sections of sham operated control spinal cords. Localization studies and examination of later intervals are in progress.

The second model selected for study was experimental autoimmune encephalomyelitis (EAE), a disease characterized by perivascular mononuclear cell infiltrates, myelin breakdown, relative sparing of axons and subsequent regeneration of myelin. Studies of growth factors and responses of astrocytes, oligodendrocytes and microglia in these demyelinating lesions have just begun.

Herpesvirus Pathogenesis and Nervous System Disease

Animal models of neurotropic human herpesvirus infection mimic many features of human disease. Thus, their systematic study provides useful insights on nervous system disease mechanisms. In autopsy tissues, certain problems in human disease may be examined directly. During FY 1992, areas of herpesvirus pathogenesis and human neurologic disease investigated were:

Host Effects of Herpes Simplex Virus (HSV) Infection in Sensory Ganglia

(i) The first quantitative evidence that HSV infection of sensory ganglia leads to neuronal death was produced. The mouse footpad model of HSV-2 (MS strain) infection was examined. In ipsilateral 4th and 5th lumbar ganglia that innervate the inoculated footpad, neuronal loss of 70% was estimated, using the best available sampling method. (ii) Last year, we showed in the same model that following acute HSV infection in the same ganglia, neuropeptide expression was selectively altered. A proportion (about 15%) of neuronal perikarya was transiently induced to express galanin, which is not detectable in normal adult ganglia. This year, this galanin response was confirmed in the mouse corneal HSV-1 model. As proportions of neurons expressing other neuropeptides (calcitonin gene-related peptide, substance P, neuropeptide Y) were not altered, this effect appears to be selective. This finding, which implies alteration in a cell program in ganglionic neurons surviving acute HSV infection, is novel, and underscores the complexity of ganglionic HSV infection. Whether this is part of a regenerative response to neuronal death or relates to the

establishment of latent HSV infection in neurons remains to be established.

Molecular Mechanisms of HSV-1 Regulation and Pathogenesis in Mouse Models

(i) Host genes which participate in the regulation of acute, latent and reactivated HSV-1 infections have not been defined. Even though many viral genes are known which affect acute replication, no viral genes have been found which regulate latent infections. In a study completed in FY 1992 it was shown for the first time that expression of a single host protein (the homeodomain protein, Hox 1.3) in transgenic mice resulted in a marked alteration in the pathogenesis of acute infection. Viral replication, spread of virus within tissues, and mortality were increased in Hox 1.3 transgenic mice. (ii) Studies are in progress to characterize the HSV-1 immediate early promoter (ICP4) in transgenic mice in the absence of viral proteins and under conditions which mimic latency and viral reactivation. These experiments should establish whether viral proteins are necessary to induce HSV-1 gene expression during viral reactivation. (iii) Studies have also begun in transgenic mice which contain a deleted form of the HSV-1 transcriptional activator protein (VP16); expression of the deleted protein in tissue culture is known to result in decreased viral replication. Strategies to interfere with viral replication using proteins which target viral regulatory genes may be important in future therapies for HSV-1 infection.

Molecular Methods to Detect HSV and Other Herpesvirus Sequences in Human Autopsy Tissues

(i) An unresolved question in neonatal HSV infection is the extent to which brain disease relates to HSV infection. Brain lesions may be extensive, yet HSV antigen is found infrequently. In current studies, polymerase chain reaction (PCR) technology permits detection of HSV DNA sequences in brain regions with histologic lesions but no detectable HSV antigen. Thus, in ways yet to be established, HSV infection may relate to brain lesions even if viral antigen cannot be detected. (ii) Initial PCR studies in archival autopsy tissues show that HSV-1, HSV-2, varicella zoster virus and cytomegalovirus DNA sequences can be amplified if DNA alterations induced by formalin fixation are limited, and that sequences of Epstein-Barr virus and human herpesvirus type 6 can be amplified if purified viral DNA is used as a template. Studies on giant cell arteritis and multiple sclerosis have been initiated.

Human Polyomavirus JC in Animal Models and in Human Neurologic Disease in AIDS and Non-AIDS Patients

We have continued to study JCV in human PML tissue, with the major discovery of two genotypes of JCV in human PML brains. Significant advances have also been made in the diagnosis of PML

from formalin-fixed, paraffin-embedded biopsy or autopsy tissues. With collaborators in other programs, we have found evidence that JCV is present in some human brains without evidence of PML. We have also continued to exploit two animal models, the neonatal hamster and the transgenic mouse in our efforts to understand the pathogenesis of JCV-induced demyelination in PML, and the basis of tumorigenesis by JCV. These findings are described in more detail below.

Two Major DNA-based Types of JC Virus Defined PML in PML Brains of AIDS and Non-AIDS Patients.

To explore the natural variation in JCV DNA sequences in PML brains, we sequenced a 610-bp region amplified from 11 PML brains from both AIDS and non-AIDS patients. This fragment, which encompasses both VP1 and T-antigen coding regions, was found to contain 20 sites of point mutations which allowed reliable classification of all JCV strains into two types, termed Type 1 and Type 2. Only in the T-antigen gene do these type-specific mutations lead to amino acid substitutions. Thus, these types are unlikely to be serologically defined, but might have subtle differences in T-antigen controlled functions. Both types have been detected in both AIDS and non-AIDS PML. Simple typing schemes with type-specific PCR primers will allow further studies of the role, if any, that JCV type plays in the outcome of JCV brain infection.

Diagnosis of JCV Infection in Fixed PML Tissues by PCR.

PCR methods of detection of JCV DNA have been adapted to formalin-fixed paraffin sections. These methods were able to detect viral DNA sequences in sections from 33 of 46 (72%) autopsy tissues examined with at least one of two sets of primers employed. While not as sensitive in fixed tissues as immunocytochemical detection of JCV capsid proteins (probably due to the effects of overfixation in some tissues) the ability to distinguish JCV from related polyomaviruses (e.g., SV40 and BKV), and eventually, to distinguish JCV Types 1 and 2 with type-specific primers, make this a valuable addition to the available approaches to PML diagnosis. We have applied this method to paraffin-embedded sections scraped from glass slides, even those that were previously immunostained with antibodies to capsid proteins. Confirmation of PML in biopsy tissue by this method allowed description of a case from Los Angeles in which PML in an AIDS patient responded dramatically to high-dose AZT therapy. Additional applications have involved virus-specific detection of JCV in PML tissue previously reported to be caused by SV40.

Evidence for the Presence of JCV in Some Non-PML Human Brains.

The high sensitivity of detection of viral DNA sequences by PCR has allowed examination of frozen sections of brains without

known CNS disease for the presence of JCV DNA sequences. More than 50% of frozen sections from these non-PML brains were found to harbor low levels of JCV DNA. Amplified DNA from the regulatory region was cloned and sequenced, and found to represent rearranged DNA resembling strains isolated from PML brains, rather than the "archetype" configuration excreted from non-PML kidney. If confirmed, these findings suggest that some cases of PML due to JCV may arise from a previously latent brain infection, and they also raise the possibility of JCV involvement in other diseases of neuroglia, perhaps triggered by immunopathologic mechanisms.

Findings in Animal Models: The Neonatally Infected Hamster

When injected intracerebrally into newborn hamsters, JCV induces brain tumor formation 4-6 months later. Studies of expression of the JCV early protein, T-antigen, have continued, with the demonstration of T-antigen expression by immunocytochemical methods in endothelial cells of such hamsters. When these animals were immunocompromised with cyclophosphamide, the expression in endothelial cells was dramatically increased, whereas T-antigen expression in nonvascular cells was not affected. Virus expression was especially pronounced at the internal granular layer-white matter junction in the cerebellum. This was previously found to be a prominent location for T-antigen expression in neoplastic foci. While the significance of these findings for human PML is not yet certain, they do focus attention on the possible consequences of endothelial cell infection by JCV, and its modulation by immunocompromising conditions.

Functional and Cytotoxic Responses of Cultured Schwann Cells

In nerve lesions involving injury to myelinated fibers, return of function depends on regeneration of transected axons and a series of interactions that they must have with Schwann cells before and during myelin regeneration. To study how regenerating axons might regulate proliferation of Schwann cells and their production of laminin, supernatants of proximal nerve segments which contain regenerating axons were prepared and added to cultured Schwann cells. Compared to those from normal nerves, supernatants 24 and 48 hr after transection increased Schwann cell proliferation significantly as measured by an ELISA for bromodeoxyuridine (BrdU) incorporation and by the numbers of anti-BrdU positive Schwann cell nuclei. The 24 hr supernatants also significantly increased laminin production, which was assessed by anti-laminin Schwann cell immunoreactivity before and after fixation and by ELISAs normalized for microculture protein amounts.

In other tissue culture experiments, changes in Schwann cell numbers and in their fine structure were studied after exposure to psychosine. Inclusions and severe, progressive alterations in mitochondria and other organelles were found before retraction of cell processes and necrosis. These findings add to the evidence

suggesting that the accumulation of psychosine in myelin-forming glial cells is cytotoxic and may have an important role in producing the myelin breakdown seen in Krabbe's disease, a severe, progressive, autosomal, recessive neurologic disease of infancy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02549-11 LENP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Herpesvirus Infections and Nervous System Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	J.R. Martin, M.D.	Medical Officer	LENP, NINDS
Others:	P. Gressens, M.D.	Visiting Fellow	LENP, NINDS
	W.J. Mitchell, D.V.M., Ph.D.	Sr. Staff Fellow	LENP, NINDS
	D.B. Henken, Ph.D.	Sr. Staff Fellow	LENP, NINDS
	H. deF. Webster, M.D.	Chief	LENP, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

2.1

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project examines nervous system diseases associated with human herpesvirus infections. These include the neurotropic herpes simplex virus types 1 and 2 (HSV-1, -2) and varicella zoster virus (VZV), as well as the other four human herpesviruses known or suspected to infect the nervous system (cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human herpesvirus types 6 and 7 (HHV-6, -7)). Where possible, experimental models are used to examine mechanisms underlying production of neural lesions. Problems of particular interest are: the role of infection with HSV, VZV and other herpesviruses in the production of CNS and PNS disease, including (i) acute encephalitis, (ii) infections during nervous system development, (iii) chronic demyelination, and (iv) mechanisms of CNS arteritis and stroke induced by neurotropic herpesviruses.

During FY 1992, studies in human autopsy tissues from HSV-infected neonates showed that, using polymerase chain reaction methods, viral DNA sequences could be detected in histologically abnormal brain regions in which HSV infection could not be demonstrated by other means. In other studies, VZV sequences were detected in formalin-fixed, paraffin-embedded human autopsy tissues with other evidence of VZV infection. Similar methods give positive results in human CMV-infected tissues, and in purified EBV and HHV-6 DNA. Studies to screen for DNA sequences of known human herpesviruses in autopsy and biopsy tissues with the diagnosis of giant cell arteritis and multiple sclerosis have been initiated.

In animal models, an HSV-2 mutant virus was used to establish a model of sublethal HSV-2 infection during nervous system development; this model is in initial stages of evaluation. In a collaborative study, a model simian varicella virus infection was completed. This is perhaps the best available model for human VZV infection, and provides useful insights into mechanisms of acute and latent VZV infection in man.

8/LENP/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01995-20 LENP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cellular and Molecular Studies of Myelin Formation, Breakdown and Regeneration		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Principal Investigator:	H.deF. Webster, M.D.	Chief LENP, NINDS
Others:	K. Tanaka, M.D.	Visiting Fellow LENP, NINDS
	Q.-L. Zhang, M.D.	Visiting Fellow LENP, NINDS
	D.L. Yao, M.D.	Visiting Scientist LENP, NINDS
	S. Abe, M.D.	Guest Researcher LENP, NINDS
	L. Hudson, Ph.D.	Unit Chief LENP, NINDS
	M. Brenner, M.D.	Special Expert LMB, NINDS
COOPERATING UNITS (if any) Laboratory of Viral and Molecular Pathogenesis, BNP, DIR, NINDS; Laboratory of Molecular Biology, BNP, DIR, NINDS		
LAB/BRANCH Laboratory of Experimental Neuropathology		
SECTION Cellular Neuropathology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	3.8	PROFESSIONAL: 3.2 OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The goal of this project is to use quantitative light and <u>electron microscopy</u> along with <u>in situ hybridization</u> and <u>immunocytochemistry</u> to study cellular and molecular mechanisms of <u>myelin</u> formation, breakdown and <u>regeneration</u>. In nerve lesions involving injury to myelinated fibers, return of function depends on regeneration of transected axons and a series of interactions that they must have with Schwann cells before and during myelin regeneration. To study how regenerating axons might regulate proliferation of <u>Schwann cells</u> and their production of <u>laminin</u>, supernatants of proximal nerve segments which contain regenerating axons were prepared and added to cultured Schwann cells. Compared to those from normal nerves, supernatants 24 and 48 hours after transection increased <u>Schwann cell proliferation</u> significantly as measured by an ELISA for bromodeoxyuridine (BrdU) incorporation and by the numbers of anti-BrdU immunoreactive Schwann cell nuclei. The 24 hour supernatants also significantly increased laminin production, which was assessed by anti-laminin Schwann cell immunoreactivity before and after fixation and by ELISAs normalized for microculture protein amounts. In other tissue culture experiments, the fine structure of Schwann cell was studied after exposure to <u>psychosine</u>. Cytoplasmic inclusions and progressive changes in mitochondria and other organelles were found at low doses; higher doses produced rapid retraction of cell processes and necrosis. These findings add to the evidence suggesting that the accumulation of psychosine in myelin-forming cells is cytotoxic and may have an important role in producing the <u>myelin breakdown</u> seen in <u>Krabbe's disease</u>, an autosomal recessive neurological disorder of infancy. Finally, morphometric, immunocytochemical and in situ hybridization studies of six cases of <u>Balo's concentric sclerosis</u> showed that this rare clinical and pathological syndrome represents an acute progressive form of <u>multiple sclerosis</u> and that the main changes seen in partially myelinated areas are those found in ongoing myelin breakdown rather than myelin regeneration. </p>		

9/LENP/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02550-11 LENP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical and Immunologic Mechanisms in Virally-Induced CNS Demyelination		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Principal Investigator:	G. L. Stoner, Ph.D.	Chief, Neurotoxicology Section
Others:	C. Ryschkewitsch, B.S.	Medical Technologist
	H.deF. Webster, M.D.	Chief
	G.S. Ault, Ph.D.	Staff Fellow
	M. Ishaq, Ph.D.	Sr. Staff Fellow
		LENP, NINDS
		LENP, NINDS
		LENP, NINDS
		LENP, NINDS
		LENP, NINDS
COOPERATING UNITS (if any) Laboratory of Molecular Oncology, Alton Ochsner Medical Foundation (O. Prakash); Department of Molecular Biology, Penn State University (R.J. Frisque)		
LAB/BRANCH Laboratory of Experimental Neuropathology		
SECTION Neurotoxicology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project concerns mechanisms of <u>demyelination</u> in human disease and continues to focus on the human <u>polyomavirus JC (JCV)</u>, the etiologic agent of <u>progressive multifocal leukoencephalopathy (PML)</u>. PML is a fatal demyelinating disease which complicates about 5% of AIDS cases, and for which there has been no treatment known. Work this year has emphasized the detection of the virus by <u>polymerase chain reaction (PCR)</u> in PML tissues, in normal human brain tissues, in brain tumors, in human kidneys, in urine in infected hamster tissues, and in transgenic mice. (Parts of this work are reported under related projects in this Section.) We found that JCV DNA could be detected in 33 of 46 (72%) of sections from PML blocks. We have also found that JCV sequences could be detected in a paraffin section which had been scraped from slides following immunocytochemical staining. Detection of JCV in that biopsy tissue allowed confirmation of suspected PML which has been successfully treated with high dose AZT. This allows reexamination of large numbers of archival PML materials, even those which have already been utilized for conventional pathological studies. In a related project, two genotypes of JCV have been demonstrated following sequencing of a 610-bp fragment comprising the VP1 gene. This information has allowed design of type-specific primers which can also be applied to archival CNS tissues in paraffin. As these two types of JCV do not differ serologically, this approach is the only means to differentiate these JCV types, the clinical significance of which is currently unknown. This work has demonstrated that PCR is a powerful technique to detect JCV DNA sequences in sections from CNS paraffin blocks--allowing confirmation of diagnosis with great sensitivity, allowing distinction from SV40 DNA sequences with virus-specific primers, and allowing typing of JCV DNA as JCV-1 or JCV-2. In collaboration with a laboratory at Penn State university, JCV DNA sequences have also been demonstrated in frozen tissues from non-PML brains without evidence of demyelination or other pathology. This finding suggests that some cases of PML may represent the reactivation of JCV which was already latent in the brain prior to the acquisition of HIV infection or another immunosuppressive condition. </p>		
10/LENP/DIR		

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 NS 02803-03 LENP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Latency and Pathogenesis of Herpes Simplex Virus in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	W.J. Mitchell, D.V.M., Ph.D.	Senior Staff Fellow	LENP, NINDS
Others:	J.R. Martin, M.D.	Medical Officer	LENP, NINDS

COOPERATING UNITS (if any)

H. Arnheiter, Visiting Scientist, LVMP, NINDS; W. Odenwald, Staff Fellow, LNC, NINDS

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

1.0

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to understand aspects of the pathogenesis of herpes simplex virus (HSV) infection in the nervous system including the mechanism by which this neurotropic virus is regulated within neurons during acute, latent and reactivated infections. A further objective is to understand the relationship between HSV and disease.

During FY 1992 studies were continued on the development of transgenic mouse models to investigate the mechanism by which HSV-1 replication is regulated by specific host and viral transcriptional regulatory proteins during nervous system infections. Two new models were developed. Three lines of transgenic mice containing the HSV-1 major immediate early promoter sequence fused to the E. coli beta galactosidase coding sequence were generated. Preliminary experiments in these mice have indicated that, under reactivation conditions and in the absence of viral proteins, host neuronal factors are sufficient to initiate HSV-1 gene expression. Five lines of transgenic mice containing a deleted form of the HSV-1 VP16 gene which is known to interfere with viral replication in tissue culture have been generated.

Experiments which were initiated in FY 1991 and completed in FY 1992 showed that in two lines of transgenic mice expressing the homeodomain protein, Hox 1.3, the pathogenesis of HSV-1 was profoundly altered. Viral spread, replication and mortality were increased in transgenic mice. Thus, for the first time it was shown that a single host protein which is a transcriptional regulator can profoundly alter the pathogenesis of HSV-1.

11/LENP/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02804-03 LENP								
PERIOD COVERED October 1, 1991 through September 30, 1992										
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Nervous System Regeneration in a Herpesvirus Model										
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small> <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Principal Investigator:</td> <td style="width: 33%;">D.B. Henken, Ph.D.</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">LENP, NINDS</td> </tr> <tr> <td>Others:</td> <td>J.R. Martin, M.D.</td> <td>Medical Officer</td> <td>LENP, NINDS</td> </tr> </table>			Principal Investigator:	D.B. Henken, Ph.D.	Senior Staff Fellow	LENP, NINDS	Others:	J.R. Martin, M.D.	Medical Officer	LENP, NINDS
Principal Investigator:	D.B. Henken, Ph.D.	Senior Staff Fellow	LENP, NINDS							
Others:	J.R. Martin, M.D.	Medical Officer	LENP, NINDS							
COOPERATING UNITS <small>(if any)</small> M.E. Goldstein, Ph.D., Senior Staff Fellow, LNC, NINDS										
LAB/BRANCH Laboratory of Experimental Neuropathology										
SECTION Cellular Neuropathology Section										
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892										
TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 1.0	OTHER: 0.3								
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </td> <td style="width: 33%; vertical-align: top;"> <input type="checkbox"/> (b) Human tissues </td> <td style="width: 33%; vertical-align: top;"> <input checked="" type="checkbox"/> (c) Neither </td> </tr> </table>			<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither					
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither								
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <p>This project investigates neural <u>regenerative responses</u> to <u>herpesvirus infection</u>. Initial studies examine the effects of <u>herpes simplex virus type-2 (HSV-2)</u> infection on host dorsal root ganglia (DRG) in a mouse footpad inoculation model, as well as the effects of <u>herpes simplex virus type-1 (HSV-1)</u> infection on host <u>trigeminal ganglia</u> in a mouse corneal inoculation model. These experimental models of peripheral infection mimic many aspects of human clinical HSV infection.</p> <p>During FY 1992, the focus of this project was on biological changes that occur in host sensory ganglion neurons as the result of viral infection. Specifically, the following issues were addressed: 1) Is there neuronal death as a result of HSV infection? 2) Does HSV infection alter normal neuropeptide production in the host sensory ganglia? 3) Can neurobiological alterations, that have been shown in other regeneration models, be identified in this system?</p> <p>Major findings are: 1) Neuronal death occurs in sensory ganglia as a result of HSV infection. 2) There is selective modulation of host cell neuropeptides during the course of infection. Galanin, normally undetectable in adult sensory neurons, is selectively expressed following both footpad and corneal inoculation with HSV-2 and HSV-1, respectively, while the neuropeptides calcitonin gene-related peptide and neuropeptide Y do not appear to be affected. 3) Other regeneration-associated markers, such as growth-associated protein, appear altered and promise to provide insight into the effects of HSV infection on the neurobiology of host ganglionic neurons.</p>										
12/LENP/DIR										

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02807-03 LENP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) JC Human Polyomavirus Infection and Tumor Induction in the Neonatal Brain		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Principal Investigator:	H.G. Ressetar, Ph.D.	Senior Staff Fellow
Others:	G.L. Stoner, Ph.D.	Section Chief
	H.deF. Webster, M.D.	Chief
		LENP, NINDS LENP, NINDS LENP, NINDS
COOPERATING UNITS (if any) Lab. of Molecular Oncology and Cellular Biology, Alton Ochsner Medical Foundation, New Orleans, LA (O. Prakash), Dept. of Molecular and Cell Biology, Penn. State Univ., PA (R. Frisque)		
LAB/BRANCH Laboratory of Experimental Neuropathology		
SECTION Neurotoxicology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	1.2	PROFESSIONAL: 1.2
		OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project investigates <u>cellular tropism</u> of the <u>human polyomavirus, JC virus (JCV)</u>, and the role that latently infected cells may play in JCV-induced disease and <u>tumorigenesis</u>. Following childhood infection, JCV remains latent in the body. In immunocompromised individuals, reactivated JCV productively infects oligodendrocytes and astrocytes causing the fatal CNS demyelinating disease, <u>progressive multifocal leukoencephalopathy (PML)</u>. While all sites of <u>JCV latency</u> have not been determined, apparent reactivation of JCV occurs in the kidney of women during pregnancy and in organ transplant recipients. </p> <p> When injected intracerebrally into newborn hamsters, JCV establishes a nonproductive infection inducing subsequent <u>brain tumor</u> formation. In the past year we have completed two main studies utilizing the hamster model of JCV infection: 1) Two double-label immunostaining methods identified JCV T-antigen in lectin-labeled blood vessels as well as Factor VIII-labeled brain endothelial cells 7 to 30 days after intracerebral injection of JCV into newborn hamsters. The number of JCV-infected endothelial cells was increased in cyclophosphamide-suppressed hamsters and this increase was most pronounced in the cerebellum in sites of subsequent tumor formation. 2) <u>Polymerase chain reaction (PCR)</u> methods were employed to detect JCV DNA in body organ samples from 6 month-old hamsters inoculated intracerebrally with JCV as newborns. High levels of JCV DNA were found in the brain, kidney and bladder of all hamsters. While variable amounts of JCV DNA were found in the adrenal, pancreas and spleen, JCV DNA was not detected in liver, testis, ovary and uterine samples. These results suggest that in the hamster, JCV persists in a tissue-specific manner 6 months after inoculation. </p> <p> A continuing study has utilized immunostaining and PCR methods to detect the presence of JC or BK polyomaviruses or SV40 monkey polyomavirus in human pediatric medulloblastomas and choroid plexus papillomas. Due to equivocal findings, further investigation is currently being performed. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02808-03 LENP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cellular and Molecular Studies of Growth Factors during Myelin Breakdown and Regeneration in the CNS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Principal Investigator: H. Webster, M.D. Others: D. Yao, M.D. X. Liu, M.D. L. Hudson, Ph.D. C. Bondy, M.D. M. Brenner, Ph.D. N. West, M.D. G. Collins, M.D.	Chief Visiting Scientist Visiting Fellow Staff Scientist Staff Scientist Special Expert Asst. Prof. Professor	LENP, NINDS LENP, NINDS LENP, NINDS LVMP, NINDS DEB, NICHD LMB, NINDS SUNY SUNY
COOPERATING UNITS (if any) Lab. of Molecular and Viral Pathogenesis and Lab. of Molecular Biology, DIR, NINDS; Developmental Endocrinology Branch, NICHD; SUNY Health Science Center, NY		
LAB/BRANCH Laboratory of Experimental Neuropathology		
SECTION Cellular Neuropathology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 3.4	PROFESSIONAL: 3.0	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The goals of this project are to study the <u>gene expression of growth factors, myelin-related proteins and other glial proteins</u> by glial cells during <u>myelin breakdown and regeneration</u>. This year, we compared relative levels of <u>insulin-like growth factor-I (IGF-I)</u> and of <u>glial fibrillary acidic protein (GFAP)</u> mRNAs and peptides produced by <u>astrocytes</u> in demyelinating lesions induced by the copper chelator, <u>cuprizone</u>.</p> <p>Hybridization studies of lesions using specific nucleic acid probes and immunostaining with cell-specific markers showed that levels of GFAP mRNA in hypertrophic astrocytes rose earlier and persisted longer during the recovery period than IGF-I mRNA levels. This suggested that in this demyelination process, astrocytic production of IGF-I and GFAP are independently regulated. Two additional models of CNS myelin breakdown and regeneration were selected for new studies of possible growth factor production by reactive glial cells. When focal lesions are produced in the dorsal columns of rat thoracic spinal cords by cryogenic injury, lesion margins are known to contain hypertrophic astrocytes and regenerating, remyelinated axons 30 - 60 days after injury (Collins and West, 1989). Dr. West produced these cryogenic lesions in groups of rats and preliminary examination of spinal cords removed and examined in LENP 7 - 14 days later showed dramatic increases in astrocytic GFAP mRNA and peptide levels and levels of IGF-I mRNA significantly above those seen in sham-operated control spinal cords. Localization studies and examination of later intervals are in progress. The second model is experimental autoimmune encephalomyelitis (EAE), a disease characterized by perivascular mononuclear cell infiltrates, myelin breakdown, relative sparing of axons and subsequent regeneration of myelin. Studies of astrocyte responses in these demyelinating lesions have just begun.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02827-02 LENP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification, Characterization, and Etiologic Role of Human Polyomavirus in Neurological Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	M. Ishaq, Ph.D.	Senior Staff Fellow	LENP, NINDS
Others:	G.L. Stoner, Ph.D.	Chief, Neurotoxicology Section	LENP, NINDS

COOPERATING UNITS (if any)

Dept. of Molecular and Cell Biology, Penn State University (R.J. Frisque)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project was undertaken to study the latency of JC virus (JCV) in human central nervous system and the possible role of the virus in neurological diseases of unknown origin. In collaboration with the laboratory of Dr. R.J. Frisque, sections of brains and kidneys were screened for the presence of JCV DNA by polymerase chain reaction (PCR). Using regulatory and coding region-specific oligonucleotide primers, more than 50% of the normal (non-progressive multifocal leukoencephalopathy (PML)) brains were found to contain low levels of JCV DNA. Cloning and sequences analysis of the PCR products confirmed the PCR findings. While the archetype strain of JCV was identified in normal kidney samples, PML kidneys and normal brains contained JCV strains resembling strains isolated from PML brain. These findings suggest JCV latency in the normal human brains. The latent virus might persist in the brain without causing demyelinating disease. The reactivation might take place after interaction with an immunocompromising agent, e.g. HIV or due to prolonged immunodeficiency, resulting in PML.

The presence of the DNA sequence of the Mad-1 strain of JCV in the brain tissues from patients with multiple sclerosis (MS) and medulloblastoma reported in the last year's annual report was found later on not to represent true JCV sequences but contamination of the tissues with pBR322 cloned JCV Mad-1 plasmid DNA from ENZO probe (used for in situ hybridization). A tri-primer PCR procedure was developed for the detection of the simultaneous presence of cloned and free viral DNA in tissue samples. The procedure was successfully employed to detect the contaminated cloned DNA in the tissues.

During amplification by PCR of the regulatory region of Mad-1 strain of JCV, we found that certain primer pairs can give rise to an artifactual deletion of one copy of a tandem repeat contained within the amplified region. The data indicated that the tandem repeats can be subject to deletion artifacts if one primer is close to the repeat unit and/or the primer binding site encompasses a potential secondary structure. In the case of repeated viral promoter/enhancer elements, these spurious products should not be confused with "archetype" sequences which naturally lack duplication.

15/LENP/DIR

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02847-01 LENP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphological and Molecular Studies of CGRP in the Gastric Wall

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	G. Jakab, M.D.	Visiting Scientist	LENP, NINDS
Others:	K. Pacak, M.D.	Visiting Fellow	CNB, NINDS
	H. deF. Webster, M.D.	Chief	LENP, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	2.0	PROFESSIONAL:	1.5	OTHER:	0.5
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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to study the distribution of the calcitonin gene-related peptide-immunoreactive (CGRP-IR) primary afferent fibers in the intact rat gastric mucosa and around experimental stress ulcers. The varicose CGRP-IR fibers run toward the luminal epithelium in the lamina propria in close connection with blood vessels and smooth muscle cells suggesting a functional, neurochemical relationship between them. The nerve fibers' branching pattern suggests the presence of columnar neurovascular regulatory units in the mucosa that are oriented perpendicular to the lumen. We found a number of CGRP-producing (probably non-neuronal) cells of at least two types located mainly near the border between the mucosa and submucosa. Since these cells are also present in variable positions following stress ulcer induction, it seems that at least two different systems, a neural and a non-neural, are responsible for the CGRP trophic support of the gastric wall. Our observations also help confirm the theory that CGRP may play a role in the development of local vasodilation, congestion and therefore in mucosal damage associated with the stress ulcers.

16/LENP/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02849-01 LENP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology of Human Polyomavirus Pathogenesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Principal Investigator:	G.S. Ault, Ph.D.	Staff Fellow LENP, NINDS
Others:	G.L. Stoner, Ph.D.	Chief, Neurotoxicology Section LENP, NINDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Experimental Neuropathology		
SECTION Neurotoxicology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Although the prevalence of benign <u>JC virus infection</u> of the kidney is very high, routes of transmission and events leading to brain pathology are not known. We sought a means of classifying and organizing viral isolates as an aid in studying such questions. Two major types of JCV have been identified. A 610-bp segment of the 3' ends of the T-antigen and VP1 genes amplified and sequenced from the brains of 11 <u>progressive multifocal leukoencephalopathy (PML)</u> patients contains 20 sites of point mutation which allow reliable classification of JCV isolates. At these 20 type-determining sites the presence of alternate nucleotides creates two distinct patterns of substitution, allowing six of the isolates to be grouped into Type 1 and the other 5 into Type 2. The <u>prototype strain Mad-1</u> was found to be <u>Type 1</u>, while the other previously sequenced strain, <u>GS/B</u>, is a <u>Type 2</u>. There are three additional type-determining sites to the early side of origin, which have allowed classification of many of the published regulatory region sequences. Only 4 of our 11 isolates had a 'crossover' to the opposite type consensus sequence, indicating a very high type specificity. These crossovers, along with random unique mutations, allow individual strains to be distinguished. No cases of multiple infecting strains were observed; however, one strain contained six crossovers, suggesting the possibility of genetic recombination in potential rare cases of multiple infection. Based on these stable point mutations, two sets of PCR primers were developed which type-specifically amplify shorter fragments that also contain type-specific restriction enzyme sites, permitting rapid and accurate classification of new isolates. Although amino acid sequence is conserved between types in the VP1 gene, several sites in the T-antigen gene cause a type-specific amino acid substitution. Comparison of each type's consensus sequence at type-determining sites to those sites in BK virus suggests that Type 2 represents the ancestral JCV sequence from which Type 1 diverged during human evolution. The regulatory regions from these strains showed roughly defined patterns of rearrangement, with individual variation unique to each strain. The observed patterns are not specific to one or the other viral type, and probably are generated by the host cell. Specific blocks of sequence are consistently preserved, and several combinations of elements apparently are viable. Usually, only one rearranged promoter form is isolated from each individual brain.</p>		
17/LENP/DIR		

ANNUAL REPORT
OCTOBER 1, 1991 THROUGH SEPTEMBER 30, 1992
LABORATORY OF MOLECULAR BIOLOGY

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Annual Report

October 1, 1991 Through September 30, 1992

Laboratory of Molecular Biology
Basic Neurosciences Program
Division of Intramural Research
National Institute of Neurological Disorders and Stroke

Franklin G. Hempel, Ph.D., Acting Chief

The Laboratory of Molecular Biology investigates the structure and function of genes in neuronal and glial cells. Among the genes studied are those that code for neurotransmitter receptors, calcium channel proteins, enzymes in neurotransmitter metabolism, and glial structural proteins. In particular the laboratory aims to characterize transcription promoter regions and the functions of transcription regulatory factors.

1. Regulation of Gene Activity in Astrocytes.

The functions revealed of astrocytes in the central nervous system now include support of growth and maintenance of neurons and oligodendrocytes, ion homeostasis, neurotransmitter metabolism, and response to injury and disease. The cell-specific mechanisms involved in gene expression in astrocytes is a major thrust in the laboratory, leading to a goal of better understanding the roles of these glial cells in normal and pathophysiologic neural function.

Efforts are directed toward analysis of the human gene encoding glial fibrillary acidic protein (GFAP), an intermediate filament protein expressed abundantly, and almost exclusively, in astrocytes. In previous reports, the isolation of human cDNA and genomic clones for the *gfa* gene has been described, as well as identification of the protein and mRNA start sites, characterization of its promoter sequences, and elucidation of the interaction of TFIID with the TATA box region upstream of the mRNA start site. Present work is focused on identifying the specific DNA sequences responsible for the cell-specific expression of the gene, and using these regions to control expression of transgenes in mice.

Using transfection of cultured cells with chloramphenicol acetyltransferase (CAT) reporter gene constructs, it was found that astrocyte-specific expression can be directed by a 2 kb 5'-flanking segment of the human *gfa* gene. This segment was found to contain two broad enhancer regions that synergistically regulate *gfa* expression. One region, called D, is located between 59 and 132 base pairs (bp) upstream of the mRNA start site, while the other region is located about 1500 bp upstream. On the basis of footprint data, this upstream region has been subdivided into A and B segments. When the A, B and D regions were juxtaposed to form a synthetic 400 bp promoter, increased levels of astrocyte-specific expression were observed. By studying the activity of various combinations of the A, B and D regions, and by also interchanging them with regulatory regions from an ubiquitously expressed SV40 gene, it has been determined that the *gfa* B region is the one most critical for cell-specific expression. Site-directed mutagenesis of this 120 bp segment has revealed that it contains several sites important for gene activity. One site, whose alteration causes a complete loss of activity, contains the consensus sequence for AP-1 binding. Gel mobility shift assays were used to show that cultured

astrocytes contain proteins of the c-Jun and c-Fos families of transcription factors that bind to this site. Furthermore, altering a single base in the AP-1 binding sequence prevented binding of these proteins and destroyed transcriptional activity in transfected cells.

Studies with transgenic mice are also being conducted in order to determine if the regulatory sites identified by transfection of cell cultures behave similarly in a living animal and to develop an astrocyte-specific expression system that will permit study of the function of various genes in astrocytes. To evaluate the function of the identified *gfa* regulatory sequences, transgenic mice carrying the bacterial β -galactosidase (*lacZ*) reporter gene under the control of the full-length (2 kb) or synthetic ABD (400 bp), promoters have now been examined for specificity of expression. The 2 kb promoter drives *lacZ* expression in astrocytes throughout the brain whereas the 400 bp promoter activity is confined primarily to astrocytes in the cerebral cortex. The up-regulation of GFAP that follows injury to the brain is seen with the 2 kb promoter but not with the 400 bp promoter. These studies demonstrate surprising heterogeneity among astrocytes, and the use of multiple regulatory elements in the control of GFAP expression.

Further studies have also been conducted to more fully examine the transcription mechanisms initiated by binding of TFIID with the TATA box in the promoter region of genes in general. At least seven general transcription initiation factors (TFIIA, TFIIB, TFIID, TFIIIE, TFIIF, TFIIJ and TFIIH) in addition to RNA polymerase II, are involved. The first step in preinitiation complex formation is TFIID binding to the TATA box in the promoter region. Subsequently, TFIIB binds to the preformed TFIID-TATA box complex and acts as a "bridging" factor to incorporate RNA polymerase II into the complex and specify the transcription initiation site. Further association of TFIIIE, TFIIG/J, and TFIIH completes preinitiation complex formation. The functional domains of TFIIB required for both binding to the TFIID-TATA box complex and for transcription initiation were analyzed. Deletion analysis showed that only the C-terminal two-thirds of TFIIB, which contains the σ -factor sequence similarities, two basic repeats and direct repeats, is sufficient for interaction with the TFIID-TATA box complex. However, an extra N-terminal 84 amino acid region, which does not contain any obvious known motifs, is required for transcription activity. Moreover, amino acid substitution analysis indicates that the second basic repeat of TFIIB is an important domain for interaction with the TFIID-TATA box complex.

Using anti-TFIID τ (a TATA box-binding component of native TFIID) immunoaffinity chromatography, 9 polypeptides (210k, 110k, 85k, 62k, 58k, 42k, 28k, 22k, 21kDa) were identified as native *Drosophila* TFIID components that are tightly associated with TFIID τ and were in agreement with the proposed large molecular weight of native TFIID. To analyze the functional activity of purified TFIID components, template DNA and other transcription factors were reconstituted with purified TFIID on an antibody-Sepharose resin. Immobilized TFIID mediated not only a basal level of transcription but upstream-stimulating factor (USF) activation as well. However, recombinant TFIID τ immobilized on antibody resin cannot mediate USF activation. These results suggest that one or more of these additional polypeptides are required as functional TFIID subunits to regulate transcription by USF in cooperation with TFIID τ .

Glutaminase (GA) and glutamine synthetase (GS) are the regulatory enzymes in the metabolism of glutamate and glutamine in the central nervous system. Glutamine synthetase catalyzes the synthesis of glutamine from glutamate and ammonia, while phosphate-activated GA catalyzes the reciprocal reaction. They are differentially

partitioned in the CNS; GS being principally localized in astrocytes, while GA is found in neurons. Glutamatergic neurons release glutamate into the synaptic cleft where it interacts with postsynaptic receptors. Signaling is terminated neither by classical reuptake into the presynaptic neuron nor by postsynaptic enzymatic degradation, but rather by diffusion from the synaptic cleft. Released glutamate is taken up by surrounding astrocytes where it is converted into glutamine by GS. The glutamine produced is then free to enter the nearby neurons where it can either reenter the neurotransmitter pool of glutamate through GA catalysis, or participate as an ammonia donor in intermediary metabolism. Due to the central roles that GS and GA play in regulating excitatory and inhibitory amino acid neurotransmitter synthesis and degradation, regulation of these enzymes at the transcriptional and post-transcriptional levels is a major research pursuit.

Previous studies in this project have isolated and fully characterized both cDNA and genomic clones of GS. Sequence analysis of the 5' flanking region containing the promoter elements has identified 3 regions of cis-regulatory importance by partial homology to: the consensus sequence for AP2, a transcriptional activator; a glucocorticoid response element (GRE); and a silencer found in the collagen type II gene. The functional importance of these sites was first demonstrated by a series of promoter deletion mutants fused to a chloramphenicol acetyltransferase (CAT) reporter gene, which confirmed the activating and inhibiting roles played by the GRE and silencer, respectively.

To confirm that the sites were responsible for the effects seen in the deletion studies, a series of site-directed mutations were made in the promoter fusion constructs. These mutations confirmed that the suspected sites were responsible for the activation and inhibition seen with the GRE and silencer sites. Furthermore, the modulator site with homology to the AP2 consensus binding site interacted with both the GRE and the silencer. Although possessing no intrinsic activity, the modulator site is required for GRE activity and in a more complex manner, the silencer. It is not known whether these interactions are due to a direct contact between the trans-acting proteins or occur through tertiary bridging factors. These activities were seen only in primary astrocyte cultures, C6 glioma cells, and the positively responding HepG2 hepatoma cell line. The site-directed mutant studies also uncovered a fourth cis-acting element with silencer-type activity which does not appear to interact with the modulator site, and whose presence was not anticipated through sequence analysis.

Nuclear extracts have been prepared from primary astrocyte cultures, HepG2 hepatoma, and HeLa carcinoma cells and used in gel retardation assays in a first attempt to characterize the interactions between cis-acting elements and the trans-acting factors which bind them. Primary astrocyte and HepG2 extracts both contain proteins which bind all three sites. HeLa nuclear extract only binds to the GRE element, suggesting a cell-type specificity in the expression of these proteins. Purified glucocorticoid receptor also binds at the GRE sequence, suggesting that it may be a trans-acting factor for this site.

In experiments to characterize glutaminase synthesis, a 4 kbp cDNA clone for glutaminase has been isolated from a library and constructed in the PCD vector. This clone contains the 3' end of the GA gene, but does not extend to the 5' end of the coding region. The ends of previous clones contain a consensus sequence for a 3' splice site, and 40 bp of noncoding, possibly intronic, sequence. The rapid amplification of cohesive ends (RACE) procedure was used to extend the clone in the 5' direction. This has resulted in the isolation of a 1.3 kbp clone which extends into

the 5' terminus of the coding region, and serves as a probe to screen a rat hippocampal library constructed in lambda gt10. The secondary screening isolated a 1.6 kbp clone which extends beyond the 5' end of the coding region.

Northern, S1 and reverse transcription followed by polymerase chain reaction (RT-PCR) have been employed to determine the developmental and tissue-specific expression of the GA gene. GA message is expressed at the highest level in brain and kidney, and at much lower levels in heart, lung, and skeletal muscle. It is entirely absent in the liver, providing a good control for the S1 and RT-PCR studies.

2. Characterization of Muscarinic Cholinergic Receptors

Muscarinic acetylcholine receptors are members of a superfamily of cell surface receptors that transduce their intracellular signals via coupling to guanine nucleotide binding regulatory proteins (G proteins). Molecular cloning studies have revealed the existence of five different muscarinic receptor genes (m1 -m5). The chemical structure of these subtypes, particularly m2, m3 and m4, is under investigation, using polymerase chain reaction (PCR)-based mutagenesis techniques to create mutant muscarinic receptors. The mutant receptors are transiently or stably expressed in a variety of mammalian cell lines, and the pharmacology of the expressed receptors is studied in radioligand binding and functional assays (e.g., changes in cAMP levels, stimulation of phosphatidylinositol hydrolysis).

Structure-function relationship studies of a large number of mutant m3 muscarinic receptors have shown that a series of threonine and tyrosine residues are critically involved in acetylcholine (ACh) binding. These residues (which are conserved among all muscarinic receptors) are positioned at a similar level one to two helical turns away from the membrane surface within different transmembrane domains (TMD), and may thus define the plane in which muscarinic agonists bind to their target receptors. Binding studies with ACh derivatives lacking the ACh ester moiety indicated that some of the conserved threonine and tyrosine residues primarily interact with the ACh ester moiety (e.g., by hydrogen bonding) to induce high affinity binding of ACh to the muscarinic receptor. In addition, functional analysis of various Thr->Ala and Tyr->Phe mutant m3 receptors showed that Thr234 and Tyr506 (numbering based on the amino acid sequence of the rat m3 muscarinic receptor) are of critical importance for agonist-induced receptor activation. These residues are located at a similar level within TMD V and VI, respectively, which are connected by the third intracellular loop (i3). Since this region is known to play a pivotal role in G protein activation, Thr234 and Tyr506 (which are conserved among all muscarinic receptors) are possibly involved in the agonist-induced conformational changes that trigger the activation of the i3 domain and the interaction with specific G proteins.

Virtually all G protein-coupled receptors contain within their hydrophobic transmembrane domains (TMD I-VII) a series of highly conserved proline and tryptophan residues. Their potential roles in ligand binding and receptor function have been addressed using the rat m3 muscarinic receptor as a model system. A series of mutant receptors in which the conserved proline and tryptophan residues were individually replaced with alanine and phenylalanine, respectively, were created and pharmacologically characterized after their expression in COS-7 cells. Whereas less pronounced changes in receptor function resulted from the Trp->Phe substitutions, dramatic changes in ligand binding affinities, Bmax values (receptor

density), and receptor function were observed with the four Pro-→Ala mutant receptors studied. These data indicate that the conserved proline residues play key roles in the maintenance of a stable protein fold and the process of ligand-induced receptor activation.

Truncated forms of the m2 and m3 muscarinic receptors (containing TMD IV and the N-terminal portion of i3) were found to be functionally inactive and no longer bound muscarinic ligands. However, when the truncated receptors were coexpressed in COS-7 cells with C-terminal receptor domains (containing TMD VI and VII and the adjacent intra- and extracellular sequences), functional muscarinic receptors were obtained. The "reconstituted" receptors displayed ligand binding and functional properties similar to those of the wild type receptors.

Each of the five muscarinic receptors displays a distinct pattern of expression throughout the central and peripheral nervous systems. The m4 receptor, for example, is primarily found in cerebral cortex and striatum where it is thought to play a major role in controlling extrapyramidal motor function. To study the regulatory genetic elements responsible for this specificity, several large genomic clones of the human m4 receptor were isolated and mapped. Three different constructs differing in the length of the 5' flanking region were injected into fertilized mouse eggs for the preparation of transgenic mice. In all three cases, mice were obtained that had integrated the human transgene into their genomes. However, only the largest construct (containing about 30 kb of 5' flanking region) was able to direct the expression of human m4 mRNA in transgenic mice. Whereas the presence of human m4 receptor mRNA in cerebral cortex and striatum mimicked the native m4 expression pattern, the presence of the human transgene in heart and skeletal muscle was an unexpected finding.

3. Voltage-Sensitive Calcium Channels in Brain

Calcium, which enters through voltage-sensitive Ca^{2+} channels present on the cell surface membrane, has been implicated as the key second messenger that regulates a number of cellular processes in the central nervous system (CNS), such as release of neurotransmitters, activation of protein kinases, and induced expression of immediate early genes. Ca^{2+} channels themselves appear to be developmentally regulated since long-term changes in electrical activity of nerve cells that mainly reflect Ca^{2+} channel functions have been observed during development. The primary structure of Ca^{2+} channels is being determined by cDNA cloning, and the regional and cellular distributions of their mRNAs and proteins are being mapped in the brain along with localization of the genes on mouse and human chromosomes.

The differential expression of brain L- and P-type calcium channel mRNAs has been investigated in adult and developing rat brain. Full-length cDNAs corresponding to the $\alpha 1$ and β subunits of rat brain L-type dihydropyridine (DHP)-sensitive Ca^{2+} channels were cloned and sequenced in an earlier work. Subsequent localization studies have shown that the DHP-sensitive L-type Ca^{2+} channel is the major subtype predominantly expressed in neuroendocrine cells.

A number of other studies suggest that the P-type channels, initially identified in cerebellar Purkinje cells, are distributed more widely throughout the brain. Expression patterns of mRNA transcripts corresponding to the $\alpha 1$ subunit of L- and P-type Ca^{2+} channels of adult and developing rat brain were investigated by *in situ* hybridization histochemistry, using specific cRNA probes. The L-type $\alpha 1$ subunit transcript was abundantly expressed in the olfactory bulb, dentate gyrus, pituitary

and pinea¹ glands, superior colliculus, and facial nucleus; whereas the P-type $\alpha 1$ subunit transcript was highly expressed in the CA3 region of the hippocampus, geniculate bodies, inferior colliculus, and cerebellum. Resolution at the cellular level disclosed differential labeling of distinct cell types in selected brain areas, suggesting that L- and P-type Ca^{2+} channels may be localized in specific subpopulations of neurons. Results further indicate that spatial localization as well as temporal expression patterns of L- and P-type Ca^{2+} channel mRNAs undergo changes during development. For instance, the P-type calcium channel transcript gradually increased from very low levels during early embryogenesis (E 6), peaked to the highest level at postnatal day (PN) 2, and decreased thereafter in adults.

To investigate the tissue-specific expression of the alternatively spliced variants of the L-type Ca^{2+} channel $\alpha 2$ subunit, the rB- $\alpha 2$ cDNA (3,823 bp) was isolated which encodes an L-type Ca^{2+} channel $\alpha 2$ subunit with apparent Mr of 123,822. The deduced amino acid sequence of rB- $\alpha 2$ cDNA is highly similar (95% amino acid identity) to that of rabbit skeletal muscle $\alpha 2$ subunit. The rB- $\alpha 2$ protein is distinct from the previously cloned skeletal muscle $\alpha 2$ subunit protein since it contains an insertion of 7 amino acid residues and a deletion of a 19-amino acid segment between putative transmembrane domains 1 and 2. Employing reverse transcription-PCR (RT-PCR) techniques, *in situ* hybridization histochemistry, and chromosomal localization studies, the rB- $\alpha 2$ and skeletal muscle $\alpha 2$ subunit transcripts were found to be variants produced by alternative splicing of the primary transcript from a single gene, and that they are differentially expressed in brain and skeletal muscle, respectively.

Two cDNA clones, BT11 and BT8, encoding the β subunit of the DHP-sensitive Ca^{2+} channel were isolated and characterized. The deduced amino acid sequence of BT11 cDNA is very similar to that of the rabbit skeletal muscle β subunit, showing 96% amino acid identity. The BT8 clone is a splice variant of BT11 with deletion of a 45-amino acid fragment. The distribution of rat DHP-sensitive Ca^{2+} channel β subunit mRNA was examined in prenatal (E16, E19), postnatal (PN0, PN6, and PN12), and adult rat brain as well as whole-body sections of E19 embryos. In adult rat brain, large amounts of β subunit mRNA were found in the hippocampus, dentate gyrus, and median habenula. A high level of DHP-sensitive β subunit transcript was already expressed at E16, and transcript levels significantly changed in several areas during development.

With respect to chromosomal localization of murine genes encoding the $\alpha 1$, $\alpha 2$, and β subunits of the DHP-sensitive, L-type Ca^{2+} channels, two of the $\alpha 1$ subunit genes (*Cchl1a1* and *Cchl1a2*) on human and mouse chromosomes were localized earlier, and the chromosomal location determined of the third $\alpha 1$ subunit gene, *Cchl1a3*, which encodes the isoform predominantly expressed in skeletal muscle. Hybridization of a rat brain cDNA probe for *Cchl1a3* to Southern blots of DNAs from a panel of Chinese hamster X mouse somatic cell hybrids suggested that this gene maps to mouse Chromosome (Chr) 1. Analysis of the progeny of an inbred strain cross-positioned *Cchl1a3* 1.3 cM proximal to the *Pep-3* locus on Chr 1.

In contrast to the $\alpha 1$ subunit which is encoded by three distinct genes located on separate chromosomes, the $\alpha 2$ and β subunits of the DHP-sensitive Ca^{2+} channels are encoded by a single gene, located on Chr 5 and 11, respectively. The rB- $\alpha 2$ cDNA probe was used to localize the $\alpha 2$ subunit gene, termed *Cchl2a* to Chr 5. Analysis of Chr 5 alleles for several genes in an interspecies cross between NFS/N and C58/J

mice shows that *Cchl2a* can be positioned at the centromeric end of the chromosome with gene order centromere - *Cchl2a* - *Il-6* - *Pgm-1*. Similarly, the gene for the β subunit is mapped on Chr 11 with gene order centromere - *Sparc* - *Cchl1b* - *Gfap* - *Pkca*. Mapping data indicate that the DHP-sensitive Ca^{2+} channel genes are apparently dispersed in the mouse genome, unlike the sodium channel whose genes are clustered on Chr 2.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02677-08LMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Gene Activity in Astrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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LAB/BRANCH

Laboratory of Molecular Biology, BNP

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.1

PROFESSIONAL:

3.7

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Astrocytes play important roles in the development and maintenance of the CNS. To understand and manipulate astrocyte function, this project addresses transcriptional control of the astrocyte-specific gfa gene that encodes the intermediate filament protein, GFAP, or glial fibrillary acidic protein.

Using transfection of astrocytoma cells with reporter gene constructs, multiple segments have been identified within the gfa promoter and upstream regions; the segments interact to control its expression. Site-directed mutagenesis is being used to pinpoint the critical specific sequences within these segments. This will be followed by isolation and study of the regulatory proteins acting at these sites.

The activity of reporter constructs is also being studied in transgenic mice. A 2,000 base pair 5'-flanking fragment of the gfa gene has been found sufficient to drive expression of a β -galactosidase reporter gene in astrocytes throughout the CNS. Deleting from this construct a gfa segment found unimportant for expression in cultured cells also produces expression exclusively in astrocytes, but activity is largely restricted to the cortex. These results indicate that astrocytes are heterogeneous in gene expression and that different regulatory regions of the gfa gene are utilized by different types of astrocytes. Projects are also underway to use the gfa regulatory sequence to express other genes of interest in astrocytes to study brain development and function and to produce models for human diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 NS 22800-04LMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Neurotransmitter Receptor Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Jurgen Wess, Ph.D.	Visiting Associate	LMB, NINDS
Others:	Roberto Maggio, Ph.D.	Visiting Associate	LMB, NINDS
	Shu-hua Yu, Ph.D.	Visiting Associate	LMB, NINDS
	Zvi Vogel, Ph.D.	Visiting Scientist	LMB, NINDS

COOPERATING UNITS (if any)

S. Gutkind, Ph.D. (LCDO, NIDR) C. Felder, Ph.D. (LCB, NIMH)
 J. Ellis, Ph.D. and M. Brann, Ph.D. (University of Vermont)

LAB/BRANCH

Laboratory of Molecular Biology, BNP

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

4.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The five muscarinic acetylcholine (ACh) receptors (m1-m5) are members of the superfamily of G protein-coupled receptors. The primary focus of this research project is to elucidate the molecular mechanisms underlying muscarinic receptor function (ligand binding, G-protein coupling, etc.) and to define the regulatory genetic elements controlling muscarinic receptor expression. Based on the high degree of structural homology found among all G protein-coupled receptors, these studies should be of general importance for the entire receptor family. Mutational analysis of the m3 muscarinic receptor has led to the identification of a series of threonine and tyrosine residues (which are conserved among all muscarinic receptors) which are critically involved in ACh binding. These residues are predicted to define a plane in the plasma membrane in which ACh binding to the muscarinic receptors occurs. Binding studies with a series of ACh derivatives suggest that some of the conserved threonine and tyrosine residues are primarily involved in the recognition of the ACh ester bond (e.g., by hydrogen bonding). In addition, two of these amino acids were found to be critically involved in agonist-induced receptor activation. Another mutagenesis study focussed on amino acid residues which are highly conserved among all G protein-coupled receptors. A series of four conserved proline residues were identified that play key roles in receptor expression, ligand binding and receptor function. Coexpression in COS-7 cells of N- and C-terminal domains of the m2 and m3 muscarinic receptors resulted in "reconstituted" receptors that displayed ligand binding and functional properties similar to those of the wild type receptors, indicating that muscarinic receptors may behave in a fashion analogous to multiple-subunit receptors. The human m4 receptor gene was expressed in transgenic mice, however, the expression pattern of the transgene in heart and skeletal muscle was not fully consistent with the distribution of the native mouse m4 receptor. Additional lines of transgenic mice are needed to elucidate the molecular mechanisms underlying this phenomenon.

Muscarinic drugs have considerable therapeutic potential in a variety of pathological conditions including Alzheimer's and Parkinson's diseases. The delineation of a detailed molecular model of the ligand-receptor-G protein interaction should allow a more rational approach toward the development of novel therapeutic agents, as well as contributing to basic understanding of cellular signal transduction processes.

9-LMB/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02825-02LMB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Transcriptional Regulation of Enzymes Involved in Excitatory Amino Acid Neurotransmission		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	John Forbes Mill, Ph.D.	Senior Staff Fellow LMB, NINDS
Others:	Hemant Purohit, Ph.D.	Visiting Associate LMB, NINDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular Biology, BNP		
SECTION Developmental Biology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	2.0	PROFESSIONAL: 2.0 OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The goal of this project is to study the structure, function, and regulation of the genes for the astrocytic enzyme <u>glutamine synthetase</u> (GS), and the neuronal enzyme <u>phosphate-activated glutaminase</u> (GA). Fusion constructs have been made between the GS promoter and the chloramphenicol acetyltransferase (CAT) reporter gene. Four regions of regulatory importance have been characterized in the GS promoter: a modulator region with homology to <u>AP2</u>, a <u>GRE</u> region, a silencer region, and a second region which inhibits transcription. Polymerase chain reaction (PCR)-based site-directed mutagenesis of these sites has demonstrated their importance in GS regulation, and the interactions between them. The modulator site is required for activity of both the GRE and silencer sites. The occult inhibitory region appears to act independently. Double-stranded oligonucleotide probes for each of these sites have been used in electrophoretic mobility shift assays (EMSA). Proteins from nuclear extracts of positively responding <u>primary astrocytes</u> and <u>HepG2 hepatoma</u> cells can bind all three sites, while those from a negatively responding cell line, HeLa, can only bind at the GRE site. The modulator site does not bind purified AP2 protein, although it contains partial sequence homology to it. The GRE site binds the DNA-binding fragment from the glucocorticoid receptor, and can compete for binding to an idealized GRE site. Biotinylated oligonucleotide probes specific for each site have been coupled to magnetic beads for affinity purification of the trans-acting factors binding to these sites. Incubation of these probes with nuclear extract from HepG2 cells has partially depleted the extracts of the silencer element, as determined by EMSA. </p> <p> Cloning the GA gene has resulted in extension of the primary clones through the rapid amplification of cohesive ends (RACE) procedure. Screening a rat hippocampal library with this probe resulted in isolation of a cDNA clone for GA which extends well into the 5' untranslated region. Reverse transcription followed by PCR (RT-PCR) has been used to determine the developmental and tissue-specific expression of the GA gene. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02828-02LMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Basis for Functional Diversities of Voltage-Sensitive Calcium Channels in Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Hemin R. Chin, Ph.D.	Senior Staff Fellow	LMB, NINDS
Others:	Hyung-Lae Kim, M.D., Ph.D.	Visiting Fellow	LMB, NINDS
	Hyun Kim, M.D., Ph.D.	Visiting Fellow	LMB, NINDS
	Hyun Ho Jung, Ph.D.	Visiting Fellow	LMB, NINDS

COOPERATING UNITS (if any)

Dr. B. Mock (LG, NCI)
Dr. C. A. Kozak (LMM, NIAID)

LAB/BRANCH

Laboratory of Molecular Biology, BNP

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	2.75	PROFESSIONAL:	2.75	OTHER:	0
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long-term goal of this project is to understand the molecular bases for the diverse structures and functions of brain voltage-sensitive calcium channels (VSCCs) using biochemical and molecular genetics techniques. Multiple types of VSCCs, (i.e., T, N, L, and P-type) are present in both excitable and nonexcitable cells and are important for excitation-contraction coupling and secretion of neurotransmitters and hormones. cDNAs corresponding to the $\alpha 1$, $\alpha 2$, and β subunits of the brain L-type dihydropyridine (DHP)-sensitive Ca^{2+} channels have been cloned and the cDNA clones encoding the $\alpha 1$ and β subunits of N- and P- types of neuronal Ca^{2+} channels have been isolated. This molecular cloning has indicated that a heterogeneous family of voltage-sensitive Ca^{2+} channels are expressed in the mammalian brain, providing structural bases for the functional diversity of neuronal Ca^{2+} channels. As a first step toward understanding the genetic bases for the diversity of brain Ca^{2+} channels, genes encoding the $\alpha 1$, $\alpha 2$, and β subunits of the VSCCs have been mapped in human and mouse genomes. With these cDNA clones as probes, the following areas are actively being pursued to elucidate molecular and genetic bases for the diverse VSCC forms and functions: 1) functional expression of VSCC by introducing full-length cDNAs for $\alpha 1$, $\alpha 2$, and β subunits into neuronal cells; 2) *in situ* hybridization histochemistry technique is being utilized to determine the spatiotemporal expression of VSCC subunit mRNAs in brain; and 3) analyses of various cDNA and genomic clones to investigate a possible role(s) of alternative splicing in generating diverse forms of VSCC present in brain. In addition, the human genomic clones will be used to identify and characterize regulatory elements in the promoter region of Ca^{2+} channel subunit genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NC 12829-02LMB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Mechanisms of Transcriptional Initiation and Activation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	Yoshihiro Nakatani, Ph.D.	Visiting Associate LMB, NINDS
Others:	Tetsuro Kokubo, Ph.D. Da-Wei Gong, Ph.D. Shinya Yamashita, Ph.D.	Visiting Fellow Visiting Fellow Special Volunteer LMB, NINDS LMB, NINDS LMB, NINDS
COOPERATING UNITS (if any) Robert G. Roeder, Ph.D. (Rockefeller University, New York) Masami Horikoshi, Ph.D. (Rockefeller University, New York)		
LAB/BRANCH Laboratory of Molecular Biology, BNP		
SECTION Developmental Biology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	4.0	PROFESSIONAL: 4.0 OTHER: .0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p>Transcription initiation factor TFIIB recruits RNA polymerase II into the promoter region by interacting with the preformed <u>TFIID-TATA box</u> complex, thus specifying the transcription initiation site. Moreover, it has been proposed that <u>TFIIB</u>, as well as <u>TFIID</u>, is a target for a transcriptional activator. Therefore, exploring the mechanism by which TFIIB interacts with the TFIID-TATA box complex is especially important to understand transcription. Through mutational analysis it has been determined that only the C-terminal two-thirds of TFIIB, which contains the σ-factor sequence similarities, two basic repeats and direct repeats, is sufficient for interaction with the TFIID-TATA box complex. However, an extra N-terminal 84 amino acid region, which does not contain any obvious known motifs, is required for transcription activity. Moreover, amino acid substitution analysis indicates that the second basic repeat of TFIIB is an important domain for interaction with the TFIID-TATA box complex.</p> <p>A reconstituted system containing a partially purified TFIID from <u>Drosophila</u> embryos stimulates transcription activity in vitro but a system containing a TATA box-binding subunit of TFIID (TFIIDτ) does not respond to trans-activators, although TFIIDτ binds to a TATA box and catalyzes basal level of transcription. Using anti-TFIIDτ immunoaffinity chromatography, 9 polypeptides (210k, 110k, 85k, 62k, 58k, 42k, 28k, 22k, 21kDa) were identified as native <u>Drosophila</u> TFIID components that are tightly associated with TFIIDτ. To analyze the functional activity of purified TFIID components, template DNA and other <u>transcription factors</u> were reconstituted with purified TFIID on an antibody-Sepharose resin. Immobilized TFIID mediates not only basal levels of transcription but upstream-stimulating factor (USF) activation as well. These results suggest that one or more of these additional polypeptides are required as functional TFIID subunits to regulate transcription by USF in cooperation with TFIIDτ.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02846-01LMB

PERIOD COVERED

January 2, 1992 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Transfer and Control in Cerebellar Granule Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Ron G. King, Ph.D. Senior Staff Fellow LMB, NINDS

COOPERATING UNITS (if any)

Evelyn Ralston (LN, NINDS)

LAB/BRANCH

Laboratory of Molecular Biology, BNP

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.75

PROFESSIONAL:

0.75

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project are to develop and use techniques to transfer genes into primary neuronal cells and to employ antisense technology to control and assess the expression of genes that may be important in neuronal development. Electroporation was used to transfect the reporter genes RSV- β -galactosidase and RSV-chloramphenicol acetyltransferase (CAT) into cerebellar granule cell cultures. RSV- β -galactosidase was expressed in both neurons and astrocytes. Assays of these cultures yielded easily detectable CAT activity. Transfection of a β -galactosidase reporter gene driven by the neuron-specific enolase promoter gave expression predominantly in neurons. Reporter gene pSV- luciferase, which should markedly increase levels of detectable activity, and phosphorothioate-modified sense and antisense oligonucleotides (oligos) are being tested. The oligos, 25 bp long and spanning the ATG site of the gene for the tau protein, are used to inhibit tau expression in order to assess the role of tau in neuronal migration and development.

ANNUAL REPORT

OCTOBER 1, 1991 THROUGH SEPTEMBER 30, 1992

LABORATORY OF VIRAL AND MOLECULAR PATHOGENESIS

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ANNUAL REPORT

Research Summary

October 1, 1991 through September 30, 1992
Laboratory of Viral and Molecular Pathogenesis
National Institute of Neurological Disorders and Stroke

Monique Dubois-Dalcq, M.D., Chief

The laboratory investigates the molecular basis of viral and other nervous system diseases. We are studying: 1) how viruses, in particular human retroviruses, papova viruses and herpes viruses, spread from extraneural sites to the nervous system, how they enter nerve cells and how their expression is regulated. The aim is to design strategies to prevent viral entry and expression in the nervous system; 2) how natural host proteins, and artificially designed antiviral factors, control viral gene expression in cultured cells and in transgenic mice. Retroviral-defective interfering particles are designed to deliver antiviral factors to specific target cells; 3) how growth factors control the generation, migration and differentiation of myelin forming cells, and how gene expression in the brain, especially myelin gene expression, is regulated at the molecular level. The aim is to understand the mechanisms underlying lineage decisions in the central nervous system (CNS), and the molecular control of gene expression in the oligodendrocyte lineage. Ultimately we want to design means to enhance remyelination in demyelinating diseases; and 4) myelin gene mutations in mice and men, and transgenic mice with insertional mutations that serve as models for human neurogenetic diseases.

MOLECULAR PATHOGENESIS

In our ongoing studies of the regulatory signals which control proteolipid (PLP) gene expression, we have found a cluster of binding sites for nuclear proteins in the cis acting elements (Hudson et al.). These motifs are shared with other myelin genes suggesting a coordination in the control of myelination. One of the trans-acting factors that recognizes some of these sites within the PLP gene is a novel member of the zinc finger class of transcription factors that is highly expressed in oligodendrocytes progenitors. This protein called MTF1 contains two widely separated sets of zinc fingers, an arrangement which would allow simultaneous binding of very distant recognition sites. Thus, this zinc finger protein may affect the structural organization of its target gene. Knocking out MTF1 gene in transgenic mice should elucidate the function that this transcription factor plays in activating PLP expression and regulating the activity of other myelin genes. As PLP, a gene characteristic of differentiated oligodendrocytes, is also expressed in early precursors, gene knock out experiments may shed some light on the function of the gene in early development. In addition, the gene for the biosynthetic enzyme glutamic acid decarboxylase, which is present in neurons in early development, is also being targeted.

In our studies of the oligodendrocytes of rodents and men (Dubois-Dalcq et al.), we have found that, besides the PDGF alpha receptor, another related receptor c-Kit is expressed in the developing rat brain and in progenitor cells at a stage preceding differentiation and myelination. Thus, it is possible that stem cell factor which binds to the c-Kit receptor has a specific effect on the growth and migration properties of oligodendrocyte progenitors. In coculture with sensory neurons, progenitors divide and become oligodendrocytes but not type 2 astrocytes. Therefore, in the intact brain, neurons may not allow the bipotentiality of progenitors observed in vitro. In the course of differentiation, oligodendrocytes express the TGF Beta 2 and 3 isoforms while only TGF Beta1 is expressed by progenitors. As TGF Beta inhibits the mitogenic response of progenitors to PDGF, the TGF Beta activity synthesized by cells in the oligodendrocytes lineage may act in an autocrine way to favor differentiation.

The development of CNS cells derived from human fetal brain is also being analyzed in vitro (Tornatore et al.). A fetal astrocyte cell line bFGF and a neuroblastoma derived factor (immortalized by SV40 T antigen) have been transfected with tyrosine hydroxylase gene and such cells will be used in transplantation of rats with striatum lesions with the hope to correct the extrapyramidal deficit.

Regeneration of oligodendrocytes has been studied in both rats and men. Astrocytes in demyelinating lesions produce a high level of IGF-1 while neighboring immature oligodendrocytes express IGF-1 receptors just before myelin genes reemerge and remyelination proceeds (Hudson et al.). The regeneration of adult human oligodendrocytes in vitro is strongly enhanced by bFGF (Dubois-Dalq et al.). Moreover, bFGF favors the emergence of preoligodendrocytes. Such cells may exist in the intact human white matter and are characterized by the expression of early developmental forms of MBP mRNAs, the PDGF receptor alpha, and the zinc finger transcription factor MTF1. These cells may constitute a small pool of precursors able to react to a demyelinating event in the adult CNS. In addition, mature oligodendrocytes stripped of their myelin sheaths have the capacity to rapidly regenerate processes in the presence of bFGF. We are pursuing our search for a growth factor that allows mitosis to occur in human myelin-forming cells. Rat sensory neurons did not provide strong mitogenic signals for these cells.

Progress has been made in the characterization of two insertional mutations in transgenic mice which map to chromosome 11 and 6, respectively (Arnheiter et al.). In both cases, the sequences flanking the inserted transgene sequences have been identified. Mice with a disruption of a gene on chromosome 11 have developmental abnormalities of the vertebra reminiscent of certain vertebral malformations in humans. Mice with disruption of a gene on chromosome 6 lack pigment cells in the skin, retina and inner ear, and are severely hearing impaired. We hope to be able to clone the gene whose mutations are responsible for the phenotype observed and to characterize human genetic diseases with similar symptomatology.

VIRAL PATHOGENESIS

A. Intracellular factors controlling viral gene expression.

a. Homeobox protein Hox1-3 and HSV-1 (Arnheiter et al)

A crucial level of regulation of HSV-1 expression occurs at the immediate early (IE) genes which may control the outcome of HSV-1 infection, i.e., lysis or latency. Hox1-3 is a nuclear protein interacting with specific sequences motives in the regulatory regions of IE genes. Transgenic mice overexpressing Hox 1-3 are markedly more susceptible to lytic HSV-1 infection. To analyze further the mechanism of action of Hox1-3 on HSV-1, these mice are crossed with transgenic mice expressing the B-galactosidase gene under an IE gene promoter.

b. Mx proteins and their relatives (Arnheiter et al). Since Mx is a member of a novel family of large GTPases, we have set out to define more precisely the biochemical and functional properties of Mx and its relative dynamin. Two different rat Mx protein have been highly purified and their structure is being examined by field emission scanning - transmission electron microscopy (in collaboration with Dr. Reese's lab). The rat Mx2 protein was shown to bind to microtubules in a nucleotide-dependent fashion similar to rat dynamin. Progress has been made toward obtaining a dynamin null mutation in mice.

c. JC papovavirus (Major et al.)

To elucidate the control of JC virus expression, we studied the ability of nuclear proteins from human fetal brain and human B cells to bind to the JCV regulatory region. NF-1 and C-jun binding sites were found to be adjacent, or overlapping with each other, in these regulatory regions. Such protein-DNA interactions are also observed with other nervous system-specific genes. JC virus has been detected in the brain and bone marrow of a patient with Wiskott-Aldrich immunodeficiency disease, suggesting that the virus can stay latent in the bone marrow.

B. Studies of infections by human retroviruses and associated infections (PML)

a. Regulation of expression of HIV-1 (Verdin et al)

These studies focus on understanding the molecular basis for HIV-1 latency and the mechanism of reactivation. To this goal, we use chronically infected T and monocytic cell lines in which viral expression can be activated by cytokines or phorbol ester. Chromatin structure of the HIV-1 genome in latently and productively infected cells is being analyzed, using micrococcal nuclease and restriction enzyme digestion of nuclei to map the boundaries of positioned nucleosomes in the 5' LTR promoter region. A nucleosome in the R.U 5 region of LTR appears displaced following induction of viral expression. In addition, new binding sites for transcription factors have been identified in this region as well as in the previously described enhancer region in the POL gene. The presence of a "repressor" nucleosome in the 5' LTR of HIV-1 may open new avenues for therapeutic intervention aiming at blocking viral expression.

b. Neurotropism of human retroviruses

Using primary culture of adult human brain (Dubois-Dalcq et al.), we have examined whether neuroectoderm derivatives can be infected by either a brain isolate or a T cell tropic strain of HIV-1. Neither astrocytes nor galactocerebroside-expressing oligodendrocytes can be productively infected in vitro. Moreover, no proviral DNA copies were found in these astrocytes derived from adult human brain. Similarly, a neuronal cell line induced to differentiate did not allow the virus to go through cycles of reverse transcription and replication. Although galactocerebroside has been described as an alternative receptor for HIV-1, we found no evidence of viral replication and expression in these cells. Moreover, ongoing infection of microglial cells in the vicinity did not affect the regeneration and survival of oligodendrocytes. Thus, the major target cell of HIV-1 in the CNS appears to be the microglia. We then studied the production of TNF α a putative toxin for oligodendrocytes, by infected microglia and found no consistent difference in the expression of this cytokine between infected and uninfected cells. Our ongoing experiments aim at determining whether HTLV-1 infects any of the CNS glial cell types present in our primary brain cultures.

In fetal glial cells, HIV-1 infection can follow a different course (Major et al). Studies of fetal astrocytes showed that a small number of cells (0.5%) can be infected by contact with virus-infected T cells. Similarly, in vivo, rare GFAP-positive astrocytes expressing HIV-1 nucleic acids (less than 1%) were seen in 4 of 8 pediatric AIDS brain tissue, raising the possibility that some developing astrocytes may be susceptible to HIV-1 infection. In fetal astrocytes transfected with HIV-1, a persistent infection occurs which can be activated by TNF-alpha and IL-1 beta (but not GM-CSF, IL-6, IL-2 and interferon) and results in rapid expression of transcripts for regulatory genes nef, tat and REV. Accordingly, the activity of LTR promoter is enhanced by TNF-alpha and/or IL-1 beta.

C. PML and AIDS (Major et al)

Ninety per cent of patients with PML contain JCV DNA in peripheral lymphocytes. This suggests that circulating lymphocytes can disseminate JC virus to the brain. JCV DNA was also found in

over 38% of HIV-1 positive patients without PML suggesting that these patients are at risk to develop the disease. The regulatory region of the JCV DNA shows complex rearrangements in the brain tissue as compared to blood.

D. Antiviral strategy and gene targeting (Schubert et al.)

We have continued the design of novel defective interfering HIV-1 particles (HIV-DIs) targeted to infect HIV-1 expressing cells. The most active HIV-DIs contain the CD4/ENV chimeric gene and a nonaribozyme directed to conserved sites in the gp120 region of HIV-1. Expression of such constructs together with HIV-1 genome in HeLa-T4 results in inhibition of HIV-1 replication partly because expression of the CD4 receptor causes down-regulation of HIV-1 env protein on the cell surface. The ribozymes should be active against most, if not all, HIV-1 variants sequenced. To generate the HIV-1 DIs, we have developed a packaging system which provides all regulatory and structural proteins of HIV-1 (except Env and nef). As the ribozyme constructs contain packaging signals, these helper constructs were able to provide the functions needed for viral assembly in HeLa cells. In other experiments, we have coexpressed the matrix protein of vesicular stomatitis virus with HIV-1 in HeLa T4 and found an important inhibition of HIV-1 replication, although this inhibition of gene expression was not specific. Other vectors will be designed and packaged in nonpathogenic viruses to deliver genes to specific CNS cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 NS02528-11 LVMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Control of Gene Expression in the Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: L. D. Hudson, Ph.D. Staff Scientist LVMP, NINDS

Others: J. Kim, Ph.D. M.S. Fellow until 4/92; presently Sr. Staff Fellow LVMP, NINDS
C. Wiese, Ph.D. Visiting Fellow LVMP, NINDS
J. Wrathall, Ph.D. Research Volunteer LVMP, NINDS
J. Berndt, B.S. Microbiologist LVMP, NINDS

COOPERATING UNITS (if any)

H. deF. Webster, Laboratory of Experimental Pathology, NINDS and J. Barker, Laboratory of Neurophysiology, NINDS.

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Myelin Regulation Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.6

PROFESSIONAL:

3.6

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Morphogenesis does not always precede the cell-type expression of genes characteristic of a terminally differentiated cell. In the nervous system, two examples of this phenomenon are the myelin-specific gene encoding proteolipid protein (PLP) of oligodendrocytes and the gene encoding the neurotransmitter biosynthetic enzyme GAD (glutamic acid decarboxylase) in neurons, both of which are actively expressed long before the appearance of the appropriately differentiated cell. How these genes are activated very early in development and what possible functions the encoded proteins assume in these precursor cells are the focus of this project. The basic approach, which is ongoing in both cases, entails constructing and evaluating transgenic mice in which the target gene or putative transcription factor(s) have been knocked out.

A long-term objective of our group has been to define the regulatory signals that control myelination, the event where oligodendrocytes extend processes that enwrap and ensheath axons. Our search for the molecular basis of transcriptional controls on myelination has identified both putative trans-acting regulatory factors and their cognate cis-acting enhancer/repressor elements necessary for expression of the prototype myelin gene proteolipid protein. One of the transcription factors that we cloned based on its ability to recognize the PLP promoter is a novel member of the zinc finger superfamily of DNA-binding proteins. The structure and expression of this protein, which appears to be restricted to cells that are the progenitors of oligodendrocytes, suggests that it may play an architectural role in organizing the PLP locus for transcriptional activation.

The isolation of transcriptional regulatory proteins permits a search for the growth factors and other molecules that are critical to the initiation and maintenance of myelin gene transcription. We have found one such growth factor, IGF-1 that may be an intermediary by which astrocytes stimulate oligodendrocytes to myelinate in vivo. This system provides a handle for identifying second messengers that may relay information to the transcriptional machinery of cells in the oligodendrocyte lineage, and therefore will enable a look at the molecular events that underly the signalling of oligodendrocytes by astrocytes.

5 - LVMP/DIR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 N5 02034-20 LVMP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Oligodendrocyte Lineage of Rodent and Man		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P. I.: M. Dubois-Dalcq, M.D. Others: R. McKinnon, Ph.D. M. Glaser Olivier Gout, M.D. R. Rusten N. Gogate, Ph.D. G. Piras, Ph.D.	Chief, LVMP Sr. Staff Fellow IPA M.S. Fellow Spec. Vol. Biol. Lab. Techn Spec. Vol. Vist. Fellow	LVMP, NINDS LVMP, NINDS LVMP, NINDS, Till July 92 LVMP, NINDS LVMP, NINDS LVMP, NINDS LVMP, NINDS Till March 92
COOPERATING UNITS (if any) Laboratory of Neurophysiology and of Neural Control, NINDS; Dr. K. Kufta, Neurosurgery Branch, NINDS and Dr. E. Friedman and M O'Connor, University of Pennsylvania, Philadelphia, PA.		
LAB/BRANCH Laboratory of Viral and Molecular Pathogenesis		
SECTION Section on Neural and Molecular Biology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 6.9	PROFESSIONAL: 4.9	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Myelin-forming cells assume an essential function in the nervous system, allowing fast conduction to occur along major nerve tracks. Loss or alteration of myelin forming cells results in neurological dysfunction such as seen in multiple sclerosis (MS). The goals of this project are to study how the development of oligodendrocytes is controlled by several polypeptide growth factors made by neurons and astrocytes in the CNS. Platelet-derived growth factor (PDGF) controls the shape, motility, mitosis and timely differentiation of oligodendrocytes progenitors in a paracrine signalling loop which is modulated by basic fibroblast growth factor (bFGF) through regulation of the PDGF receptor alpha on these cells. A related receptor c-kit is also expressed on progenitors at a discrete stage preceding differentiation. Neurons are signalling progenitors to become oligodendrocytes and not type 2 astrocytes. A later precursor - reacting with the O4 antibody - is multipolar, much less migratory, and give rise to resting stem cells which persist in the adult CNS. When moving along the oligodendrocyte pathway, the cells synthesize different isoforms of TGF beta which can modulate their own growth in an autocrine manner.</p> <p>In the adult human white matter a small population of glial cells express genes characteristic of an early stage of oligodendrocyte differentiation such as the PDGF receptor alpha and myelin basic protein (MBP) mRNAs containing exon 2 information. Cultures of adult human white matter also contains immature cells characterized as preoligodendrocytes. bFGF is a potent stimulator of regeneration of processes by adult human oligodendrocytes and can cause dedifferentiation in these cells. We are presently investigating whether such cells can be induced to divide and differentiate by purified factors and neuronal derived signals in vivo and in vitro. These studies may pave the way to the design of strategies to enhance remyelination in demyelinating diseases. Another factor crucial in successful myelin repair is the ability of cells with remyelinating potential to migrate toward lesions. We found that grafted glial cells in rodents are able to migrate through the normal white matter toward a demyelinating lesion and are now investigating the specific signals that trigger this migration.</p>		
6 - LVMP/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 ZO1 N5 02852-01 LVMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuro and Gliogenesis in the Developing Human Brain and Uses for Transplantation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Carlo S. Tornatore, M.D.	Senior Staff Fellow	LVMP, NINDS
	Walter Atwood, Ph.D.	Staff Fellow	LVMP, NINDS
	Judith Boston, Ph.D.	Biologist	LVMP, NINDS
	Blanche Curfman, B.S.	Microbiologist	LVMP, NINDS
	Eugene O. Major, Ph.D.	Section Chief	LVMP, NINDS
	Renee G. Traub, B.S.	Microbiologist	LVMP, NINDS

COOPERATING UNITS (if any)

Surgical Neurology Branch, NINDS
 Department of Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School.

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Section on Molecular Virology and Genetics

INSTITUTE AND LOCATION

National Institute of Neurological Disorders and Stroke

TOTAL STAFF YEARS:

1.9

PROFESSIONAL:

1.5

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The developing human central nervous system consists of pluripotent cells which mature into astrocytes, oligodendrocytes and neurons. We have been examining human fetal brain tissue from gestational ages ranging from 8 weeks to 22 weeks inclusive to determine at which gestational ages each of these differentiated cells can be identified. The constituent elements of the developing central nervous system can be separated from one another by mechanical methods allowing study of individual cellular components. This also allows the production of highly purified cultures of fetal neurons or astrocytes which can be used in cell culture models for HIV-1 or other neurotropic infections. These brain cell cultures can also be useful in testing transplantation protocols for therapy of neurodegenerative disorders. We have previously developed an immortalized human fetal astrocyte line (SVG) and have implanted the cells into rat brain. We have also further modified these cells by insertion of a tyrosine hydroxylase gene construct which is amplified by replication of its ORI sequences using the constitutively expressed SV40 T protein. These cells, SVG-TH, could potentially serve as an alternative to neural grafts of primary tissue in transplantation studies.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

ZO1 NS 02790-04 LVMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Insertional Mutations in Transgenic Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Heinz Arnheiter, M.D.	Visiting Scientist	LVMP, NINDS
Others:	Susan Skuntz, B.S.	Biologist	LVMP, NINDS
	Colin Hodgkinson, Ph.D.	Visiting Fellow	LVMP, NINDS
	Ellen Meier, Ph.D.	Sr. Staff Fellow	LVMP, NINDS

COOPERATING UNITS (if any)

Dr. Kenneth Grundfast, M.D., LMO, NIDCD; Dr. Tachibana, M.D., LMO, NIDCD; Dr. Jorgen Fex, M.D., Chief, LMO, NIDCD; Dr. N. Jenkins, Dr. N.G. Copeland, Dr. K. Moore, ABL Basic Res. Program, NCI.

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Viral Pathogenesis Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.4

PROFESSIONAL:

1.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transgenic mice with insertional mutations constitute a potentially rich source of molecularly tagged important genes. We have selected two insertional mutations for closer scrutiny. Mice with an insertion on chromosome 11 (map position 58) display a mild developmental abnormality of the vertebrae characterized by vertebral fusions and split vertebrae particularly in the thoracic and lumbar regions. This phenotype is reminiscent of that created by mutations in *pax 1*, a vertebrate transcription factor, whose gene, however, is on chromosome 2. It is conceivable that the interrupted gene on chromosome 11 is one of the elusive target genes of Pax 1. We have identified a piece of genomic DNA flanking the inserted transgene. The corresponding sequence is expressed on a 2.8 kb mRNA found in adult tissues such as muscle and heart. Currently, we are identifying a second piece of flanking DNA that might allow us to map the extent of any deletion that may have accompanied the insertion. We will then proceed to identify the transcript of the region of chromosome 11 that is responsible for the phenotype. A second insertion occurred into the genetically defined *mi* locus on mouse chromosome 6. Mutations in *mi* are characterized by the triad, loss of coat pigment, small red eyes, and hearing deficiency. The common denominator of these defects may be the underdevelopment, or loss, of functional melanocytes throughout the body. In fact, analysis of the inner ear of these transgenic mice has revealed that the so-called stria vascularis is free of melanocytes, a phenomenon that is associated with degeneration of the outer hair cells. We have identified two pieces of genomic DNA flanking the inserted transgenes. These sequences are present on a single genomic fragment of 6.6 kb and define an RFLP in another line of mice with a mutation at *mi*. One of these probes detects on Northern blots a ~7.0 kb mRNA found in adult skin and melanocyte cell lines, and not in many other organs. Thus, this mRNA likely represents a transcript of the *mi* gene.

The importance of the analysis of transgenic insertional mutations not only lies in the analysis of developmentally important genes but also in the potential to molecularly characterize human genetic diseases with similar phenomenology. The vertebral abnormality defined by the chromosome 11 insertion is reminiscent of certain human vertebral malformations, and the *mi* mutation is a model for particular forms of human Waardenburg syndrome characterized by hearing deficiency and pigment abnormalities.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02698-07 LVMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology of Mammalian Homeodomain Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Heinz Arnheiter, M.D. Visiting Scientist LVMP, NINDS

COOPERATING UNITS (if any)

W. F. Odenwald, Ph.D., Staff Fellow, LNC, NINDS; Shang-Ding Zhang, Ph.D., Vis. Fellow, LNC, NINDS; W. J. Mitchell, D.V.M., Ph.D., Sr. Staff Fellow, LNEP, NINDS

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Viral Pathogenesis Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland, 20892

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Herpes simplex virus is an important human pathogen associated with diseases ranging from mild recurrent genital or facial lesions to fatal infections of newborns. It has been postulated that *in vivo*, the regulation of the immediate early (IE) genes of HSV-1 may control the severity of an infection and whether the infection will be lytic or remain latent. In order to define intracellular host and viral factors that might control IE gene expression *in vivo*, we have focused on proteins capable of interacting with the TAATGARAT regulatory regions of IE genes. We have found earlier that *in vitro*, the mouse homeodomain protein Hox 1.3 binds TAATGARAT motifs. To test whether over-expression of Hox 1.3 would alter HSV-1 pathogenesis, we generated transgenic mice in which the level of Hox 1.3 is regulated by virus infection (by way of responding to a virus/interferon-inducible promoter). Such transgenic mice are indeed markedly more susceptible to lytic HSV-1 infection, most likely because Hox 1.3 protein is induced in the very cells that become infected upon exposure to HSV-1. To study whether this increased susceptibility to HSV-1 is due to a direct action of Hox 1.3 on the IE gene regulatory region, we have generated additional transgenic mice that express the bacterial β -galactosidase reporter gene under an IE gene promoter and we crossed these mice with the Hox 1.3 transgenics. In double transgenic mice, we will induce the Hox 1.3 protein with interferon and test whether this induction would result in transactivation of the reporter gene. In addition, we have generated transgenic mice that harbor a cDNA for a truncated form of the viral VP16 protein that is incapable to transactivate IE genes but capable to compete with wild type VP16. These lines of mice have been expanded on a HSV-1 susceptible background and will become ready to test for alterations in the course of HSV-1 infection and latency in the near future. This project will be continued by Dr. Mitchell in LNEP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 Ns 02742-06 LVMP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanisms of Viral Pathogenesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Heinz Arnheiter, M.D. Others: Ellen Meier, Ph.D. Ravi Kambadur, Ph.D. Colin Hodgkinson, Ph.D. Helen Lee Hellmich, Ph.D.	Visiting Scientist Senior Staff Fellow Visiting Associate Visiting Fellow IRTA Fellow	LVMP, NINDS LVMP, NINDS LVMP, NINDS LVMP, NINDS LVMP, NINDS
COOPERATING UNITS (if any) S.B. Andrews, Ph.D, LN, NINDS; P.E. Gallant, Ph.D., LN, NINDS, T.S Reese, M.D., LN, NINDS		
LAB/BRANCH Laboratory of Viral and Molecular Pathogenesis		
SECTION Viral Pathogenesis Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 3.4	PROFESSIONAL: 3.4	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> A novel family of large GTPases ($M_r = 70,000-100,000$) includes the following proteins: the interferon-inducible <u>Mx proteins</u> of vertebrates; the constitutive, microtubule-binding protein <u>dynamin</u> of <u>Drosophila</u> and vertebrates; the <u>mitochondrial protein MGM1</u> of yeast, and the cytoplasmic protein <u>VPS1/SPO15</u> of yeast. Despite striking sequence similarities, these proteins differ widely in their biological functions as defined by genetic models. The goal of our studies is to define more precisely the biochemical and functional parameters of the vertebrate members of this family of proteins. </p> <p> In some species, such as rats or mice, Mx proteins show activities against viruses that are of no importance to those species, suggesting the proteins' primary function may not be to inhibit viruses. In support of this suggestion, we have found that the anti-VSV protein <u>Mx2 of rats binds microtubules</u> in vitro in a nucleotide-dependent fashion, similar to rat dynamin. Thus, Mx2 may have a role in cellular processes involving microtubules, and the antiviral action of Mx2 may merely be a byproduct of such a cellular role. The precise function of dynamin is not known for vertebrates but appears to be in <u>endocytosis</u> (for instance at the neuromuscular junction) in <u>drosophila</u>. In order to <u>genetically define</u> the function of dynamin, we plan to generate a dynamin <u>null-mutation</u> in the mouse. To this end, we have isolated genomic cosmid and lambda phage clones corresponding to the mouse dynamin gene. </p> <p> Purification of rat Mx proteins derived from E. coli allowed us to initiate a detailed <u>biochemical analysis</u> of their enzymatic properties. The purified preparations also have been used to generate <u>polyclonal rabbit antisera</u> and to obtain initial <u>structural</u> information by low temperature, field emission scanning-transmission electron microscopy (Dr. Brian Andrews, LN). These will be useful to elucidate the mechanism by which viruses are inhibited. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 01983-21 LVMP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Pathogenesis of JC Virus and Progressive Multifocal Leukoencephalopathy		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I. Eugene O. Major, Ph.D. Walter Atwood, Ph.D. Katherine Conant, M.D. Blanche Curfman, B.S. Linda Durham, M.S. Carlo S. Tornatore, M.D. Renee G. Traub, B.S.	Sect. Chief, Staff Fellow Sr. Staff Fell. Microbiologist Biologist Sr. Staff Fell. Microbiologist	LVMP, NINDS LVMP, NINDS LVMP, NINDS LVMP, NINDS LVMP, NINDS LVMP, NINDS LVMP, NINDS
Kei Amemiya Ph.D., CRADA, Igen, Inc. Jurgen Harms, Special Volunteer, Med Student, Goettingen, Germany		
COOPERATING UNITS (if any) Department of Neurology, University of Miami, Department of Neurology, VA Hospital, Washington, D.C. Medical Neurology Branch, NINDS. Animal Health Care Section, NINDS.		
LAB/BRANCH Laboratory of Viral and Molecular Pathogenesis		
SECTION Section on Molecular Virology and Genetics		
INSTITUTE AND LOCATION National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Md.		
TOTAL STAFF YEARS: 3.1	PROFESSIONAL: 2.0	OTHER: 1.1
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Investigations of the pathogenesis of <u>JC Virus</u> induced <u>progressive multifocal leukoencephalopathy</u> (PML) focus on mechanisms of latency of JCV in human tissues, viral activation and transport to the brain and transcriptional control of viral gene expression. Experiments are conducted at the clinical, biological and molecular levels. Using PCR analysis, JCV from suspected latently infected lymphocytes in bone marrow has been found in peripheral lymphocytes in more than 90% of PML patients, particularly individuals with AIDS as the underlying immune disorder. JCV DNA was also found in greater than 50% of immune suppressed individuals without PML identifying them at risk for the development of PML. DNA sequence analysis of the regulatory region from several PML patients demonstrated the prototype (two 98 base pair repeat units) sequence arrangement in peripheral blood but a greatly rearranged DNA sequence in the brain. JCV DNA was also found for the first time in a Wiskott/Aldrich syndrome patient in biopsies of bone marrow and brain, and in bone marrow and kidney tissues taken three years prior to PML at the time of therapeutic splenectomy. This unique case study of PML highlights the observation of JCV latency in marrow cells of the marrow. These results suggest that JCV could be spread to the CNS by a hematogenous route, possibly through a B lymphocyte vector. To examine molecular control of viral expression, nuclear proteins from human fetal brain and human B cells were examined for their ability to bind to the JCV regulatory region. A protein factor(s) from both of these sources was able to bind several sites which contain the recognition sequence for a NF-1 protein. A c-jun like factor was also able to bind the regulatory region. The NF-1 and c-jun binding sites were either adjacent or overlapped each other. Examination of the regulatory regions of many other genes expressed in the brain such as MBP, GFAP, PLP, S100B, NF-L, and pro-ENK revealed that they also appear to contain adjacent binding sites for NF-1 and an activator protein immediately upstream from the mRNA start site. These results suggest that they share common factors which regulate these genes in a tissue-specific manner.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02830-02 LVMP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of HIV Transcription In Vitro and In Vivo		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P. I.:	E. Verdin, M. D.	Senior Staff Fellow LVMP, NINDS
Others:	A. El Kharroubi, Ph.D. C. Van Lint P. Paras, B.S.	Visiting Fellow LVMP, NINDS Special Volunteer LVMP, NINDS Biologist LVMP, NINDS
COOPERATING UNITS (if any) Arsene Burny, Ph.D., University of Brussels, Brussels, Belgium		
LAB/BRANCH Laboratory of Viral and Molecular Pathogenesis		
SECTION Section on Neural and Molecular Biology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	4	PROFESSIONAL: 2.5 OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The objective of this project is to define the molecular mechanism controlling the <u>expression of HIV-1</u> in infected cells. Our studies focus on understanding the molecular basis for latency and the mechanism of <u>reactivation from latency of HIV-1</u>. We are using, as a model, several chronically infected cell lines which harbor the virus in a "latent" state. Low level viral expression present in basal conditions can be activated in these cell lines after treatment with cytokines (TNF-α, GM-CSF) or phorbol esters. Our previous work examining the chromatin of HIV integrated in these cell lines identified several DNase I hypersensitive regions in the 5' and 3' LTR and in the <u>pol</u> gene. In addition, a new hypersensitive site was noted in the 5' LTR upon induction of viral expression. We have used micrococcal nuclease and restriction enzymes digestion of purified nuclei to map the boundaries of positioned nucleosomes in the 5' LTR. These studies demonstrated that the U3 region of the 5'LTR is nucleosome-free and therefore accessible for transcription factors to bind. The R-US region seems protected by a <u>nucleosome</u> which is removed following induction of viral expression. This nucleosome could play a role in the maintenance of latency and its removal could be the first step in activating transcription. Binding sites for transcription factors have been identified in this 'inducible' region and are being characterized. The intragenic DNase I hypersensitive site has also been examined at higher resolution in vivo with micrococcal nuclease and restriction enzymes identifying a 300bp nucleosome-free region. This region, which precisely maps where an enhancer was previously identified, has been studied in vitro by DNase I footprinting, methylation interference and gel retardation assays. Seven sites for DNA-binding proteins have been identified and characterized. The role of each of these sites in the activity of the enhancer is being defined by mutagenesis and transfection studies. The significance of these studies lies in the potential relevance for HIV-1 latency and reactivation. The identification of a repressor nucleosome in the 5'LTR of HIV-1 could open new therapeutic opportunities to suppress replication. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02789-04 LVMP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurotropism of Human Retroviruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P. I.: M. Dubois-Dalcq, M. D. Others: Jia Min Zhou Donald Gilbert Susan Wilt	Chief, LVMP Vist. Associate, Techn. Howard Hughes Fellow IRTA Fellow	LVMP, NINDS LVMP, NINDS LVMP, NINDS, Till July 92 LVMP, NINDS, starting July 92
COOPERATING UNITS (if any) Dr. K. Kufta, Neurosurgery Branch, NINDS, Dr. E. Friedman and Dr. Michael O'Connor, University of Pennsylvania, Philadelphia, PA.; Eric Verdin, LVMP, NINDS.; Igor Koralnik and Veffa Francchini, Laboratory of Tumor Cell Biology, NCI.		
LAB/BRANCH Laboratory of Viral and Molecular Pathogenesis		
SECTION Section on Neural and Molecular Biology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.3	PROFESSIONAL: 1.3	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The human retroviruses HIV-1 and HTLV-1 can both infect the CNS causing the AIDS psychomotor complex and tropical spastic paraparesis, respectively. The goal of this project is to analyze the cell and molecular basis of the <u>neurotropism</u> of these human retroviruses. To this end, we have used primary cultures of adult human brain which can be enriched in <u>microglia</u>, astrocytes and oligodendrocytes. Our studies on HIV-1 infections in vitro indicate that the major target cell of the virus in the brain is the microglia, a cell of the monocyte-macrophage lineage. Neither astrocytes nor galactocerebroside-expressing <u>oligodendrocytes</u> can be productively infected in vitro. Moreover, no proviral DNA copies were found in these astrocytes derived from adult human brain. Similarly, a <u>neuronal cell line</u> induced to differentiate does not allow the virus to go through cycles of reverse transcription and replication. Thus, HIV-1 entry and/or expression in neuroectoderm derivatives appears to be a rare event or inefficient process. We have shown before that only macrophage tropic strains replicate in microglia and that HIV-1 entry in microglia is mediated through the CD4 receptor. The region of the HIV-1 glycoprotein encompassing the <u>V3 loop</u> is critical for efficient entry in microglial cells as anti V3 antibodies can block infection of our brain cultures. Thus, therapeutic approaches to stop virus spreading to the brain could be targeted to the <u>CD4 binding site</u> or the specific V3 loop sequences of microglia tropic isolates.</p> <p>In our current experiments with <u>HTLV-1</u>, we are using similar approaches to determine in which cell of the nervous system this retrovirus can enter and replicate.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02851-01 LVMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HIV-1 Infection in Human Fetal Brain Cell Cultures and Pediatric AIDS Brain Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Eugene O. Major, Ph.D.	Section Chief	LVMP, NINDS
	Kei Amemiya, Ph.D.	CRADA	Igen, Inc.
	Walter Atwood, Ph.D.	Staff Fellow	LVMP, NINDS
	Katherine Conant, M.D.	Senior Staff Fellow	LVMP, NINDS
	Karen Meyers	Biologist	LVMP, NINDS
	Carlo S. Tornatore, M.D.	Senior Staff Fellow	LVMP, NINDS

COOPERATING UNITS (if any)

Pediatric Branch, NCI, NIH
Department of Pathology, National Children's Hospital, Washington, D.C.

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Section on Molecular Virology and Genetics

INSTITUTE AND LOCATION

National Institute of Neurological Disorders and Stroke

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Mechanisms that explain how HIV-1 causes damage in the pediatric brain have focused on alternative explanations; either HIV-1 can induce toxins from infected macrophages and/or cause a low level infection in select populations of glial cells. We have established a useful model of HIV-1 infection in human fetal brain cell cultures to address this question. Through either infection with virions or transfection with proviral DNA, human fetal astrocytes, which do not express the CD4 receptor for HIV-1, quickly develop a non-cytopathic but productive infection which gradually diminishes to a persistent infection. Persistence of the viral genome is maintained in an integrated state in the host chromosome without expression at the RNA or protein levels. However, HIV-1 can be activated by factors provided by CD4 + human lymphocytes. Cytokines TNF-alpha and IL-1 beta are also able to activate the latent HIV-1 genome following transfection, to again progress into a productive but limited infection. Other cytokines known to induce HIV-1 from human monocyte cells such as GM-CSF, IL-6, IL-2, and interferon do not activate HIV-1 from astrocyte. Further evidence of the specificity of these cytokines comes from experiments using an HIV-1 LTR CAT vector transfected into astrocytes. CAT activity is highest in cells treated with TNF-alpha and IL-1 beta even in the absence of the viral transactivator, tat protein. Within 24 hrs of cytokine addition to persistently infected astrocytes, HIV-1 mRNAs for nef, tat, and rev proteins can be identified. The nef transcript appears to be the most abundant HIV-1 transcript. Examination of brain tissues from 12 pediatric AIDS cases also revealed astrocytes, GFAP staining, with positive hybridization to HIV-1 radiolabeled probes in 4 cases. In several of these sections, there was no evidence for HIV-1 antigens, p24 and gp 41. These results suggest that astrocytes may harbor an unexpressed HIV-1 proviral DNA that can be activated in the brain through cytokines. TNF-alpha and IL-1 beta are reported to be present in AIDS brain tissue in high concentrations. Further study of the molecular mechanism of transcriptional control of HIV-1 and its clinical correlates in pediatric AIDS encephalopathy are currently in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTENT - RAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02818-03 LVMP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pseudotypic Defective Interfering HIV Particles as an Antiviral Therapy for AIDS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	M. Schubert, Ph.D.	Section Chief LVMP, NINDS
Others:	A. C. Banerjee, Ph.D. C.-J. Chen, Ph.D. S.-Y. Paik, Ph.D., Ph.D. G. G. Harmison II, M.S.	Senior Staff Fellow Visiting Associate Visiting Fellow Chemist LVMP, NINDS LVMP, NINDS LVMP, NINDS LVMP, NINDS
COOPERATING UNITS (If any)		
LAB/BRANCH Laboratory of Viral and Molecular Pathogenesis		
SECTION Viral Replication Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD		
TOTAL STAFF YEARS: 4.3	PROFESSIONAL: 4.1	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The main purpose of this project is to develop an <u>antiviral strategy</u> directed against HIV-1 which would delay the onset of AIDS. Unlike the use of antiviral drugs, our strategy is a biological approach which employs novel <u>defective interfering HIV-1 particles</u> (HIV-1 DIs) targeted to infect HIV-1-expressing cells. Replication and gene expression of these DI particles are totally dependent on the regulatory and structural proteins provided by HIV-1. We anticipate that progeny DI particles will not be pathogenic. They will be able to spread the interfering genes specifically to other HIV-1 infected cells or to cells HIV-1 would normally infect. Unlike naturally occurring defective interfering particles of other virus groups, these DI particles will be dominant and they will uniquely use HIV-1 as their host. The anticipated spread of the interfering genes within the monocyte/macrophage population throughout the body will be slow and it will occur during the asymptomatic phase of the HIV-1 infection. Important is that the particles should also target cells in the <u>central nervous system</u>. It is our hope that the DI particles will slowly limit or down-regulate the HIV-1 load, and thereby delay or possibly even prevent the onset of AIDS.</p> <p>We have developed six generations of these HIV-1 DI genomes. Coexpression of each of these constructs with HIV-1 resulted in a dramatic inhibition of HIV-1 replication in HeLa T4 cells. Interference was caused by the HIV-1 Tat dependent expression of a <u>chimeric CD4/env receptor protein</u> encoded by the HIV-DI genome. Expression of the receptor caused a downregulation of the HIV-1 Env protein on the cell surface. The HIV-1 DIs did not inhibit HIV-1 gene expression, but they caused virus progeny to be less infectious. Novel catalytic RNAs (<u>multitarget-ribozymes</u>) were developed which were designed to target and to specifically cleave at ten different, highly conserved sites in the envelope region of the HIV-1 RNA. These multitarget-ribozymes showed unique properties as compared to single cleavage ribozymes. They were highly functional even when they were part of a large transcript. Based on sequence analyses they should be active against most if not all HIV-1 variants presently sequenced. From the fourth HIV-1 DI generation on, these multitarget-ribozymes were part of the HIV-1 DI genomes. We have shown that these HIV-1 DIs interfere with the replication of HIV-1 by two different mechanisms: 1. the complex formation between the HIV-1 Env with the chimeric receptor and 2. the specific in vivo cleavage of HIV env mRNA by the multitarget-ribozyme. In fact, the genomic RNA of the HIV-1 DI itself functioned as a multitarget-ribozyme. To generate the HIV-1 DIs in the absence of HIV-1, a new HIV-1 packaging DNA was developed. Cotransfection with the HIV-1 DI DNAs resulted in the efficient assembly of HIV-1 DIs. The packaging efficiencies of the HIV-1 DI genomic RNAs No. 4 through No. 6 are currently under investigation. Cocultivation of HIV-1 DI expressing cells with cells persistently infected with HIV-1 has currently been started. This will allow a first evaluation of the effectiveness of our <u>antiviral strategy</u> in tissue culture. It is our hope that the HIV-1 DIs will perform safely as outlined in our strategy and that they can be used in a biological <u>antiviral therapy</u> in the future.</p>		
15 - LVMP/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02791-04 LVMP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Replication and Pathogenesis of Enveloped Viruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	M. Schubert, Ph.D.	Section Chief LVMP, NINDS
Others:	S.-Y. Paik, Ph.D., Ph.D. A. C. Banerjee, Ph.D.	Visiting Fellow Senior Staff Fellow LVMP, NINDS LVMP, NINDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Viral and Molecular Pathogenesis		
SECTION Viral Replication Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	1.0	PROFESSIONAL: 0.9 OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The broad range of this project covers several aspects in basic molecular virology and pathogenesis of enveloped viruses. The purpose is to identify and to understand the molecular mechanisms in viral entry, gene expression pathogenesis and assembly. The ultimate goal of these studies is the development of nonpathogenic viruses as highly specific gene delivery systems for the central nervous system. For the delivery of specific genes to the brain, possibly through the cerebral spinal fluid, enveloped viruses and particularly retroviruses are preferred viral candidates, however, nonenveloped viruses such as the nonpathogenic adeno-associated virus should also be evaluated.</p> <p>During the past FY, efforts by the Viral Replication Section toward these goals were limited. The main part of the study focused on the mechanism of pathogenesis by one envelope virus, vesicular stomatitis virus (VSV). The possibility was explored to direct the pathogenic effect of VSV towards inhibiting another virus (HIV-1). We had earlier reported that the matrix protein M of VSV alone is responsible for at least one cytopathic effect caused by the virus: the disruption of the cytoskeleton. The matrix protein was coexpressed with an infectious clone of HIV-1 in permissive HeLa T4 cells. This resulted in a dramatic inhibition of HIV-1 replication. The inhibition caused by the M protein was not specific for HIV-1, since the expression of other marker genes, all expressed by RNA polymerase II, was also inhibited. In fact, even the expression of a gene from a vaccinia virus recombinant was inhibited in the cytoplasm. The dramatic inhibition of HIV-1 by the M protein, did however, result in an effective protection of the entire cell population which was permissive for HIV-1. The potential use of the M gene for an intracellular immunization of cells against HIV-1 is currently under investigation.</p> <p>In addition, a chimeric glycoprotein between the VSV G protein and the HIV-1 Env protein was assembled by precise gene fusion. The goal was to change the ecotropic HIV-1 Env protein to an amphotropic glycoprotein, like the VSV G protein, by combining the receptor binding function of VSV G with the membrane fusion function of the HIV-1 Env protein. More extensive studies will be required, however, to be able to maintain the full dual function of such chimeric glycoproteins. A detailed knowledge of these structures will be essential for the specific targeting of cells in the central nervous system. Such systems could potentially be used for the therapy of neurological disorders and diseases such as Alzheimer's and Parkinson's diseases, multiple sclerosis, brain tumors, AIDS dementia, etc.</p>		
16 - LVMP/DIR		

ANNUAL REPORT

October 1, 1991 through September 30, 1992

Laboratory of Neural Control, Basic Neurosciences Program, Division of Intramural Research
National Institute of Neurological Disorders and Stroke

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ANNUAL REPORT
October 1, 1991 through September 30, 1992
Laboratory of Neural Control
Basic Neurosciences Program, Division of Intramural Research
National Institute of Neurological Disorders and Stroke

Robert E. Burke, M.D., Chief

Introduction

Research in the Laboratory of Neural Control (LNL) is devoted to studies of the central and peripheral neural mechanisms involved in the control of movement in vertebrates. Our work emphasizes analysis of neural organizations at the level of the spinal cord and those regions of the brainstem and cerebral cortex that project directly to the spinal cord in vertebrates, ranging from birds to primates. Spinal cord development is examined using in vitro techniques, ranging from tissue culture systems to whole chick embryos, and include extra- and intracellular electrophysiological and optical recording techniques. Although the fundamental mechanisms of neurite formation and guidance are being studied in dissociated neurons maintained in tissue culture, most of the in vitro work involves explants of spinal cord and/or brainstem of chick embryos or the lumbosacral spinal cord of neonatal rats. Interactions among motoneurons, motor units, and interneuronal circuits are being studied using single unit electrophysiological methods in acute in situ preparations of adult cats. The organization of cortical motor control systems is studied in primates, primarily using chronic recording techniques in awake, behaving animals. Neuroanatomic, histochemical and immunocytochemical, and mathematical modeling approaches are used as adjuncts to these basically physiological studies. LNL also includes a section devoted to studies of peripheral nerve regeneration after injury, primarily with light and electron microscopic anatomic techniques. Finally, members of the Laboratory continue to be involved in studies of neural prostheses that utilize state-of-the-art techniques to develop systems that can benefit neurologically-handicapped individuals.

Present Organization

During FY-1992, the staff of the LNL included fourteen professional scientists (five permanent senior scientists, one special expert, and eight post-doctoral fellows). The permanent staff also consists of eight full-time permanent support personnel (two engineers, one computer programmer, three biological technicians, one histology technician, and one laboratory secretary).

The FY-1992 research effort in LNL can be described under five general headings:

1. Electrophysiological, morphological, and theoretical studies of the properties of individual spinal cord neurons, using dissociated cell cultures or in vitro explants from embryonic chick or neonatal rat, or in vivo preparations in adult cats.

Project Numbers: Z01 NS 02079-19 LNL
Z01 NS 02160-18 LNL
Z01 NS 02787-04 LNL
Z01 NS 02788-04 LNL

2. Electrophysiological, morphological, and theoretical studies of functioning neuronal circuits within the spinal cord and brain stem of immature and mature animals with particular emphasis on the mechanisms involved in the generation and control of intrinsic motor patterns.

Project Numbers: Z01 NS 01686-24 LNL
Z01 NS 02079-19 LNL
Z01 NS 02787-04 LNL

3. Studies of the discharge properties of individual neurons in the primate motor cortex and supplementary motor area (SMA) during performance of voluntary movements.

Project Number: Z01 NS 01688-24 LNL

4. Studies of the mechanisms of injury repair in mammalian peripheral nerves following axotomy and the role of the blood-nerve barrier in this process.

Project Number: Z01 NS 02254-16 LNL

5. Activities concerned directly with the development of new methods for making contact with the central nervous system (CNS). Almost all the activities under this rubric serve to support ongoing research projects within LNL, including the development of a functional visual prosthesis for application to the human visual cortex. Virtually all staff members of LNL participate in one or more aspects of this project, both as an adjunct to their own research and as a way to share the fruits of their efforts with other staff members and projects. Many of the techniques and instruments developed in LNL are new and without commercial counterpart. In such cases, LNL staff continue to provide assistance to other scientists at NIH and at other institutions around the world who request information and advice about specific data acquisition and processing problems.

Project Number: Z01 NS 01688-24 LNL

Project Summaries

Section on Neural Mechanisms

The Section on Neural Mechanisms is devoted to studies of individual neurons and of neuronal circuits that are involved in the control of movement in the brain and spinal cord of adult, fully mature mammals (mainly cats and nonhuman primates). Electrophysiological and neuroanatomic methods are used to acquire data from experimental animals. When appropriate, analytical and numerical computational models are then formulated, based on selected experimental data sets. The questions of immediate interest concern the role of synapse distribution and dendritic morphology on the processing of synaptic information in individual spinal cord cells (primarily motoneurons), and the organization of neuronal circuits in the brain and spinal cord that operate to control the flow of information from sensory receptors to motoneurons during reflex and voluntary movements in mammals.

Dendritic architecture and the processing of synaptic information: In virtually all CNS neurons in the mammalian brain, most of the synaptic input to individual neurons is distributed over the dendritic tree, which contains more than 90 percent of the receptive membrane in most types of CNS neurons. For many years, this laboratory has been involved in studies of the interaction between synaptic action and the electrotonic architecture of postsynaptic dendrites in the control of information flow within individual neurons. The most accessible synaptic system available for direct study of this problem in the mammalian CNS is the monosynaptic contacts made by group Ia muscle spindle afferents on alpha-motoneurons. We have an ongoing series of theoretical studies related to the effect of dendritic and synaptic morphology on the electrotonic properties of dendritic trees and the resultant influence on the flow of synaptic currents in branching tree structures. Most of this work during FY 1992 has dealt with questions about dendritic morphology.

The anatomic complexity of neuronal dendrites has hampered attempts to undertake comparative studies of dendrites in different neuronal species, or in a given neuron type subjected to experimental manipulation (e.g., axotomy). Over the past two years, we have developed a relatively simple stochastic growth model that reproduces the statistical properties of alpha-motoneuron dendrites, based on the idea that the minimal description of complex objects can be embodied in the set of rules that can reproduce those objects. The main parameters of the model are probabilities of branching or terminating that depend primarily on local branch diameter and, to a lesser extent, on the distance from the soma. These, together with an algorithm to specify the diameters of daughter branches at branching points permit the

simulation of complete dendritic trees. All parameters are derived directly from experimental data and the degree of fit between simulated and real dendrites is used to guide the extraction of additional dependencies from the original data. This process has revealed dependencies that were not evident on simple inspection of the data, and provide a concise description of motoneuron dendrites.

The model parameters are ratios that can be applied to neurons regardless of their size. This is a powerful feature that allows detailed quantitative comparisons to be made between the fundamental factors that control dendritic morphology in different species of motoneurons. We previously reported that the dendritic trees of completely reconstructed alpha- and gamma- motoneurons can be described with the same basic model, but the model parameters display different dependencies on local diameter and distance from the soma in the two groups. Both samples have now been enlarged (7 gastrocnemius and 11 gamma motoneurons) and the comparisons have been extended to include 7 soleus motoneurons from material labeled in LNL and in the Department of Anatomy, Karolinska Institutet, Stockholm. In addition, we have received data on 6 phrenic motoneurons in adult cats from Dr. William Cameron of the University of Pittsburgh. All five groups of adult motoneurons display systematic differences, mainly in the probabilities of branching and terminating. These comparative studies have shown the remarkable sensitivity of dendritic morphology to what seem like relatively small differences in model parameters.

These comparative studies will continue with examination of the parameter differences in motoneurons labeled at different post-natal ages in kittens and cats, one set representing hindlimb motoneurons from the Stockholm group and the other phrenic motoneurons in the cervical spinal cord from the University of Pittsburgh. Conventional data analysis methods have suggested that considerable remodeling of dendrites takes place during early postnatal development in the cat. Our approach offers the possibility to isolate those factors that are most important in controlling such remodeling.

The model system described above is based on Monte Carlo simulations of dendritic architecture. Dr. Marks has developed a more general, analytical model that predicts the average dendritic structure for a given stem diameter, given any set of branching and terminating probabilities. This further abstraction of dendritic morphology encapsulates the initial data set for gastrocnemius alpha-motoneurons with reasonable fidelity using only three parameters, which promises further insights into the key factors that control dendrite shape and size. Comparative studies with the other data sets available are planned. In addition, we intend to extend the present model to simulate the three-dimensional anatomy of motoneuron dendrites. It is hoped that the development of this model extension will reveal the most efficient ways to assess the very difficult problem of measuring how dendrites fill three-dimensional space.

Electrotonic properties of neuronal dendrites: We have maintained a long-standing interest in the electrotonic architecture of neuronal dendrites. The morphological studies cited above are a logical outgrowth of this interest. During FY 1992, studies in this area included compartmental modeling of the electrophysiology of three gamma-motoneurons provided by Dr. Robert Fyfe of the University of North Carolina, which were studied electrophysiologically before labeling with horseradish peroxidase (HRP). Using an approach developed in LNL several years ago, we have estimated the specific resistivities of the somatic and dendritic membrane (R_m and R_{md} , respectively), assuming reasonable values for specific cytoplasmic resistivity and membrane capacitance. As in the alpha-motoneurons studied earlier, the results indicate that R_m is significantly smaller than R_{md} in gamma-motoneurons, possibly due (at least in part) to artifactual leakage conductances that result from conventional sharp micropipettes. Interestingly, the small gamma-motoneurons do not appear to be more vulnerable than the larger alpha cells, in that the ratios, $\beta = R_m/R_{md}$, were of the same order of magnitude estimated for the latter. However, the average values of R_{md} ($52.3 \pm 8.6 \text{ K}\Omega\text{-cm}^2$), respectively) for the three gamma-motoneurons studied were larger than the mean estimates for alpha cells ($16.8 \pm 3.3 \text{ K}\Omega\text{-cm}^2$), while the average for R_m ($273 \text{ }\Omega\text{-cm}^2$) were the same.

The comparisons of dendrites in different types of motoneurons suggest that various neuron groups may be optimized for different features. For example, the dendrites of gamma-motoneurons are generally thinner and less profusely branched than those of alpha cells of hindlimb muscles but they are equally long or slightly longer. The estimates of R_{md} now at hand for both cell groups suggest that electrotonic attenuation of distal synaptic inputs are comparable in both. The morphological model allows

us to create dendrites with a variety of anatomic features that match those of other neuron groups to explore questions such as the dendritic shapes that minimize synaptic potential attenuation while maximizing dendritic territory.

The organization of excitatory segmental interneurons in the cat spinal cord: This subproject utilizes cats, either anesthetized or after decerebration, with destruction of the supratentorial brain. This animal has been used as the ideal model system for work on the anatomy and physiology of the spinal cord for over a century and there is thus a wealth of detailed information about the cat spinal cord that serves as the basis for the design and interpretation of new experiments. The neural mechanisms that control movement are inferred from data obtained in reduced, immobile preparations.

Our recent work has concentrated on the organization of excitatory segmental interneurons that project directly to alpha-motoneurons, about which there exists relatively little detailed information. We have emphasized studies of input systems that reliably produce short-latency excitation in particular groups of motoneurons, especially those that innervate the flexor digitorum longus (FDL) muscle. Past work has shown that large, low-threshold afferents from the dorsal versus ventral surfaces of the foot produce EPSPs with minimum central latencies consistent with disynaptic connection to FDL motoneurons. The SP and PLNT EPSPs are modulated differentially during fictive locomotion (rhythmic patterned motoneuron discharges in decerebrate, paralyzed animals that mimic those found intact, freely walking animals), indicating that these two skin pathways, which innervate contiguous regions on the distal hindfoot, project to motoneurons through separate and distinct sets of last order excitatory interneurons. The patterns of locomotor modulation of SP and PLNT EPSPs illustrate the precise organization of spinal interneurons and fit quite well with the activity patterns of the FDL muscle found in earlier work in this laboratory in intact, freely-moving cats, suggesting that the same sets of interneurons in the cutaneous reflex pathways may be utilized by the spinal central pattern generator (CPG) for locomotion that is presumed to be involved in normal voluntary stepping in the behaving animal. These pathways also receive convergent excitatory control from the descending corticospinal (pyramidal) and rubrospinal systems.

During FY 1992, we completed the first phase of a study of mono- and disynaptic EPSPs in many hindlimb motoneurons, including FDL cells, produced by rapidly conducting fibers that can be electrically excited by stimulating the medial longitudinal fasciculus (MLF) in the brainstem. Our results indicate that this system does not converge onto interneurons in the SP or PLNT pathways. The segmental interneurons in the disynaptic MLF pathway are thus separate from those in the excitatory cutaneous pathways studied earlier. Nevertheless, like the latter, interneurons in the disynaptic MLF pathway are facilitated by stimulation of descending pyramidal tract axons, as well as after stimulation of the mesencephalic locomotor region (MLR) that produces fictive stepping in decerebrate animals. In addition, disynaptic MLF EPSPs exhibit marked modulation during fictive stepping, indicating that interneurons in this pathway receive convergent input from the CPG. In addition, the patterns of modulation during fictive stepping are different in flexor and extensor motoneurons, with facilitation of disynaptic MLF EPSPs during the respective phases of stepping when the motoneurons are normally active. The only exception to this rule was found in FDL motoneurons, in which MLF EPSPs are facilitated in during the extension phase of stepping, in contrast to the behavior of the cutaneous EPSPs in these cells. These findings show that there are at least two distinct sets of disynaptic MLF interneurons that can be differentially controlled by the CPG, one projecting to extensor and the other to flexor motoneurons. We are currently attempting to locate the segmental interneurons in the MLF pathway to motoneurons, in order to examine their individual behavior patterns.

Spatial facilitation experiments such as described above provide important clues to the organization of spinal interneurons. However, direct recording from interneurons identified as to input sources and target neurons is required to fully answer questions about their functional role(s). For example, spatial facilitation provides evidence of convergence of synaptic inputs onto common interneurons but cannot indicate whether a given input source can control the pathway in the absence of other inputs. In the case of last-order excitatory interneurons with convergence from cutaneous afferents and the CPG, the synaptic organization could be viewed either as a reflex system modulated by the CPG (the most common interpretation) or as a set of interneurons that distribute CPG output to motoneurons in patterns that

reflect peripheral sensory information. The latter viewpoint is the more interesting and challenging, but it depends on experimental demonstration that interneurons with the appropriate input organization participate in autonomous locomotor activity without peripheral input. We have been trying for several years to gather such data but have been unsuccessful thus far because of the extreme technical difficulty of the experiment. Nevertheless, such attempts will continue.

For over a century, the in situ cat spinal cord has been the classic preparation for investigating spinal circuitry but it has a number of disadvantages. Recently, the in vitro preparation of neonatal rat spinal cord has come into vogue for studies of spinal cord pharmacology and intrinsic neuron properties. We have attempted to assess whether detailed studies of spinal circuitry are possible in this preparation, to permit exploration of issues such as CPG organization that are very difficult to attack in the in situ cord. This work is still in development.

Cortical mechanisms of voluntary motor control: Dr. Edward Schmidt, a member of the Section on Neural Mechanisms, has for many years studied the behavior of cortical neurons in non human primates in relation to various features of voluntary motor performance in operant conditioning situations. Dr. Schmidt and his coworkers have been very successful in developing chronic recording techniques for recording from individual cortical neurons in primates that are alert, comfortable, and performing highly trained movements to secure desired food and juice rewards. He has succeeded in using magnetic resonance imaging (MRI) to enhance the accuracy of recording chamber and electrode placements and has developed sophisticated data collection and analysis methods that utilize readily available microcomputers to control experimental paradigms and to collect and display data on-line for immediate evaluation during experiments.

Work with awake primates has centered on evaluating the activity patterns of individual neurons in the supplementary motor area (SMA) in macaques. Neurons in this region project directly to the spinal cord as well as to the primary motor cortex (SI) but their role in movement control is not well defined. Positron emission tomography (PET) in human subjects has shown that the SMA is active when subjects think about performing a complex movement, and is coactive with the primary motor cortex during actual performance. Although ablation of the SMA has little effect on simple movements, it does affect performance of bimanual tasks. Previous work in this laboratory showed that unilateral and bilateral cooling of the SMA did not interfere with the accurate performance of highly trained wrist flexion-extension movements that are triggered by a visual signal. More complex motor tasks have now been devised, including bimanual movements that are either symmetrical or mirror image, in order to investigate whether SMA neurons may be involved in the execution of complex movements. Primates are currently being trained to perform such relatively difficult tasks in a stereotyped way, in order to facilitate correlation with neuronal firing patterns.

The yield of information from chronic recording in the cortex is highly dependent on the techniques available to sample the cell population. The more individual neurons that can be recorded simultaneously, the greater the potential yield of usable data. In past work, multiple "map-pin" electrodes, designed and fabricated in LNLC, have been used for this purpose, in addition to conventional signal metal microelectrodes. Dr. Schmidt has continued to evaluate new electrode systems designed to provide multiple contacts for recording or intracortical microstimulation of the brain. The most recent system under test involves printed circuit technology in which six or more noble metal electrode contacts are spaced at 200 μm intervals along a silicon shaft are used to sample neurons at specific depths within the cortex. As with any new design, a wide variety of problems have been encountered with such electrodes, including excessive lead stiffness, difficulties with making contact between the printed circuit and the flexible lead wires needed for transcutaneous connection, and gradual deterioration of signal/noise ratios of unknown origin. The last problem has responded to brief passage of non-gassing currents through the electrode contacts. The principle of using printer circuit technology for making chronic electrodes remains valid and promises important advantages for both research and, in the longer term, clinical applications. However, much remains to be done to perfect a practical system.

Development of a practical visual prosthesis: This laboratory has, for many years, been active in exploring the possibility of using intracortical microstimulation as the basis for a visual prosthesis to aid

blind human subjects. Preliminary studies using intracortical microstimulation through "map-pin" electrodes in sighted volunteers undergoing occipital craniotomy for other reasons showed that perceptual "phosphenes" (points or small spots of perceived light) were generated at stimulating currents well within the range previously shown to be safe. The next step was to test whether intracortical stimulation is also effective in generating visual percepts in human subjects who had been blind for prolonged periods. Experience with the intraoperative tests made it clear that the subtlety of the effects produced and the need for precise quantitation of perceptual events required testing in a human subject capable of verbal reports. It was impossible to envision an animal test that would give the needed information.

Accordingly, LNLC staff members then developed a collaborative project to develop a feasible visual prosthesis, involving LNLC, the Surgical Neurology Branch, and the Division of Fundamental Neurosciences. The intracortical electrode design (based on the LNLC map-pin electrodes) were successfully tested in a primate implant and the final electrode arrays for human implantation were fabricated in LNLC. After extensive discussions with a variety of experts, as well as with a panel of blind patients to get a true sense of "user" opinion, a protocol for a human implant study was approved in January, 1991 after extensive technical and ethical review by NIH and FDA. After rigorous screening, a volunteer who had been blind for 22 years because of glaucoma was chosen for the study. The patient was fully informed of all risks and of the fact that this first implant would function only temporarily at best. Because of the unusual and highly invasive nature of the study, a special review panel was convened by the Director of NIH to examine the protocol and its anticipated risks and benefits. Following approval by this panel, 38 intracortical electrodes were implanted in the right primary visual area and connected to specially fabricated transdermal electrode leads. There were no intra- or post-operative complications of any sort. Daily testing of the system went on for a period of almost four months, during most of which the subject lived in a normal environment outside the hospital. At the end of that time, the transdermal leads and three map-pin electrodes were removed; the patient has experienced no sequelae.

The subject experienced reproducible phosphenes during stimulation of 34 of the 38 implanted electrodes, using currents well within the range known to produce no local tissue reaction in primates. A great variety of stimulation parameters were explored to find the optimum pulse durations, frequencies, and train durations that maximized percepts and minimized percept fading. Patterns of simultaneous phosphenes were generated by stimulating multiple electrodes. The patterns moved with the eyes through most of the eye movement range. Inadvertent breakage of the intracranial fine wire leads limited the number of working electrodes but the results obtained in this first patient clearly indicate that intracortical microstimulation is a feasible approach for a practical visual prosthesis. Modifications to the electrode lead design and development of a strain relief connector fixed to the skull are now underway, in order to prepare for a second, more permanent implant in a different patient volunteer.

Techniques for making contact with the nervous system: LNLC has maintained an identified project associated with the development of techniques to make contact with the functioning nervous system. Historically, this was done because of the laboratory's involvement with neural prostheses. The project has continued because of recognition that the development of novel recording and stimulation methods is essential to research on functioning neural systems in vertebrates. The work of our engineering staff is essential to the success of fundamental research projects within LNLC, as well as being instrumental in the collaborative effort to develop a visual prosthesis.

Section on Developmental Neurobiology

The Section on Developmental Neurobiology was established in FY 1989, under the direction of Dr. Michael J. O'Donovan. Its primary aim is to analyze the properties of spinal cord neurons and interneuronal networks during embryonic development, using an in vitro preparation of the chick spinal cord as the model system. This preparation exhibits spontaneous motor patterns that are qualitatively similar to those emitted by intact embryos and offers the possibility of studying, in great detail, the maturation of basic neuronal circuitry that produces a natural motor behavior. A variety of approaches is in use to examine this question, including some that are quite novel. The accessibility of this preparation

and the ability to maintain it in vitro offers great promise for analysis of normal motor pattern generation in a vertebrate nervous system.

Real-time optical imaging of neuronal activity: Dr. O'Donovan has successfully developed a real-time imaging system to detect changes in intracellular calcium concentration during stimulated or spontaneous motor patterns in chick embryos. Initially, the calcium indicator Fura-2AM was used with video-enhanced fluorescence microscopy and computer image processing to record the patterns of active moto- and interneurons during pattern formation. Many spinal neurons, including both motoneurons and interneurons, exhibit phasic changes in calcium fluorescence during motor output bursts, and these action potential-dependent calcium transients appear to be synchronous in all cell groups, within the time resolution of the optical system (video frame rate = 16 ms). The disadvantages of Fura-2AM are that the dye is loaded into cells by bath application, limiting loading to superficial cells. Loading also seems to be age-dependent and decreases in efficiency with embryonic age. Evidence developed during FY 1992 suggests that the observed intracellular calcium transients are primarily due to calcium influx through low- and high-threshold calcium channels, activated by action potentials. However, it is still unclear whether release from intracellular stores plays an additional role.

During FY 1992, Dr. O'Donovan's group has begun to use other calcium indicator dyes, including Fura or Calcium Green conjugated to dextran. Calcium Green produces more intense activity-dependent fluorescence changes but lacks the capacity to quantitate intracellular calcium changes that are present with Fura. Dextran conjugates have shown good retrograde transport when applied to axons in spinal roots or when injected into the spinal cord. Retrograde labeling offers the advantage that the axonal trajectories of labeled cells are defined, so that particular groups of neurons can be labeled. When distances are relatively short (up to 15 mm), there is sufficient transport by diffusion so that the indicators remain activity sensitive in labeled cell bodies. Theoretical studies in collaboration with Dr. Marks have indicated that diffusion is probably the main mechanism by which optically active indicators reach the cell bodies. Optical imaging studies have been initiated of the activity of propriospinal neurons, labeled by dye deposition in the ventrolateral white matter tracts, during motor pattern generation. There have also been some pilot studies of back-labeled neurons in the embryonic brainstem after labeling from the spinal cord.

Electrophysiological studies of pattern formation: Lesion experiments have shown that the spinal region essential for pattern generation is the ventrolateral part of the gray matter, including the motor nuclei and the region immediately dorsal to it. Retrograde labeling studies have also shown that propriospinal neurons with axons in the ventrolateral white matter are very important in coordinating the synchronous activity observed over lumbosacral segments. Both excitatory and inhibitory synaptic actions are apparently involved, since blockers of both types of amino acid transmitters affect synchronized drive potentials in motoneurons. Most of the cell bodies of the propriospinal neurons are found dorsomedial to the lateral motor column, in a region already demonstrated to be optically active during pattern generation. The roles of another group of active interneurons located contralaterally near the central canal, and of recurrent excitation among motoneurons, during pattern generation are presently being assessed. Optical imaging experiments have been extended through collaborations with several groups outside NIH, including one in England, to examine pattern generation in amphibian embryos, larval lampreys, and in the neonatal rat spinal cord.

The use of whole-cell patch-clamp recording has been extended to recording from spinal interneurons in chick embryo. One object of this work is to examine whether some interneurons exhibit intrinsic, voltage-dependent rhythmicity that could form the basis for rhythm generation. Although a few cells have been found with such properties, the majority do not. Additional pharmacological studies are needed in order to define the extent of potential intrinsic rhythmicity.

Whole-cell patch-recording has enabled further definition of the nature of synaptic conductances in motoneurons during pattern generation. Previous work showed that GABA produces depolarizing PSPs with marked conductance increases particularly in embryonic flexor motoneurons, with reversal potentials in the neighborhood of -40 mV. Agonist and blocker applications have shown that glycine and its blockers do not cross-react with GABA_A receptors, but the converse has been much more difficult to investigate.

The complex effects produced by glycine and GABA blocking agents on motor patterns do not appear to be due nonspecificity but further work is needed to clarify this issue.

Neurite formation in cultured neurons: An additional project in the Section on Developmental Neurobiology concerns studies of the initial events in neurite outgrowth in dissociated chick dorsal root and sympathetic ganglion neurons maintained in tissue culture. Phase contrast microscopy combined with time lapse video imaging is used to follow the development of individual neurites that form when cells are grown on polyornithine or laminin substrates, and when targets in the form of other neurons or microbeads coated with various substrates are available in the vicinity. The ability of artificial beads to promote neurite formation indicates that interactions with specific external receptors is not necessary in this process. Neurons are not inherently polarized and neurites can form at any site on the cell periphery, without regard to the initial location of cytoplasmic constituents.

Filopodia contain actin microfilaments but not microtubules or neurofilaments. When a filopodium contacts a permissive target, cytoplasmic extrusions containing microtubules develop rapidly; this process appears to depend on an actin microfilament motor in the cell cortex, because it is disrupted by cytochalasin B, which disrupts actin-based motility mechanisms. Microtubules appear to enter the developing neurite as preformed polymers, because it is not prevented by inhibitors of tubulin polymerization. The mechanism of cytoplasmic extrusion into a developing neurite can be studied by observing the movements of small beads attached to the surface, which serve as indicators of movements of the actin motors located in the cell cortex. The movement of cytoplasmic components and the external target coated beads suggest the existence of considerable tensile force during cytoplasmic extrusion, which can draw a target bead toward the cell body when the bead is mobile, or draw cytoplasm into the neurite when the bead is not mobile.

Interference reflectance microscopy has been used to examine the relation between neurite growth and the adhesion of the cell membrane to various substrates. Neurons growing on polyornithine display tight adhesion to the substrate over most of the filopodium length. This is associated with slow growth and little tendency to form complete neurites. In contrast, adhesion to laminin substrates appears to be looser and neurite outgrowth is favored under these conditions. When outgrowth occurs on polyornithine substrates, it is associated with lifting of the neurite from the substrate. These observations suggest that the density of neurite adherence to substrates is an important factor that controls the probability and location of outgrowth.

Section on Neuronal Regeneration

The Section on Neuronal Regeneration is devoted to studies of the factors that promote the regeneration of injured peripheral nerve fibers and the mechanisms that inhibit regrowth of sensory nerve fibers into the central nervous system after dorsal root injury. Work on peripheral nerve regeneration has used rodent models while that on sensory regrowth into the CNS is being done in pigeons, which have a particularly favorable anatomical situation.

Mechanisms of repair following injury to peripheral nerves: The major focus on this research is to elucidate the mechanisms by which peripheral nerves are repaired following crush or nerve section. One aspect has been continued analysis of the cellular events that occur within nerve "cables" that form when the two ends of the sectioned sciatic nerve are placed into the ends of a silicone tube with a space between them. Recent work has shown that cables form even when the proximal nerve stump is ligated to prevent axonal growth. Under such conditions, the cables contain Schwann cells enclosed in basement membranes and arranged in longitudinal columns. Thus, the presence of axons is not necessary for Schwann cell proliferation and organization but this takes place only when the chambers are filled with dialyzed plasma; it does not occur with buffer-filled chambers.

A second focus of work is on techniques that enhance survival of nerve grafts that have been frozen. Successful cryoprotection of nerve grafts is an essential element in developing banks of frozen human nerves to permit reconstruction of badly damaged nerves after limb trauma in patients. Excellent viability after thawing from liquid nitrogen temperatures has been found in rat nerves treated with a mixture of

formamide and dimethyl sulfoxide. Implantation of frozen nerves into conspecific rats supported normal axonal regeneration, and this was also possible in allografts after immunosuppression. Further work with large nerves, including human, is underway.

Experiments designed to determine why normal olfactory neurons can successfully enter the CNS to reinnervate their normal targets while other peripheral sensory neurons cannot do so have continued in FY 1992. This work is done in the pigeon, which has a favorable arrangement of olfactory and trigeminal nerve branches that lends itself to both self- and cross-reinnervation experiments. Current work has focussed on the effect of nerve section on the ultrastructure of the olfactory nerve itself. Olfactory axons degenerated after axotomy but were not phagocytosed, and there was no invasion of the nerve by blood-borne cells. The supporting, or ensheathing, cells of the olfactory nerve retained their linear arrangements after axotomy, surrounded by basement membrane, to provide tubes through which regenerating axons can progress. Axonal regeneration through these structures will now be studied after self- and cross-reinnervation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01686-24 LNLC																								
PERIOD COVERED October 1, 1991 through September 30, 1992																										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Motor Control Systems in the Spinal Cord																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: R.E. Burke, M.D.</td> <td style="width: 40%;">Chief</td> <td style="width: 20%;">LNLC, NINDS</td> </tr> <tr> <td>Others: M.J. Bak</td> <td>Electronics Engineer</td> <td>LNLC, NINDS</td> </tr> <tr> <td>G.M. Dold</td> <td>Engineering Tech.</td> <td>LNLC, NINDS</td> </tr> <tr> <td>M.K. Floeter, M.D., Ph.D.</td> <td>Staff Fellow</td> <td>LNLC, NINDS</td> </tr> <tr> <td>M. Manley</td> <td>Biological Lab. Tech.</td> <td>LNLC, NINDS</td> </tr> <tr> <td>G.N. Sholomenko, Ph.D.</td> <td>Visiting Fellow</td> <td>LNLC, NINDS</td> </tr> <tr> <td>J.-P. Gossard, Ph.D.</td> <td>Special Volunteer</td> <td>LNLC, NINDS</td> </tr> <tr> <td>Y. Kawai, Ph.D.</td> <td>Special Volunteer</td> <td>LNLC, NINDS</td> </tr> </table>			PI: R.E. Burke, M.D.	Chief	LNLC, NINDS	Others: M.J. Bak	Electronics Engineer	LNLC, NINDS	G.M. Dold	Engineering Tech.	LNLC, NINDS	M.K. Floeter, M.D., Ph.D.	Staff Fellow	LNLC, NINDS	M. Manley	Biological Lab. Tech.	LNLC, NINDS	G.N. Sholomenko, Ph.D.	Visiting Fellow	LNLC, NINDS	J.-P. Gossard, Ph.D.	Special Volunteer	LNLC, NINDS	Y. Kawai, Ph.D.	Special Volunteer	LNLC, NINDS
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J.-P. Gossard, Ph.D.	Special Volunteer	LNLC, NINDS																								
Y. Kawai, Ph.D.	Special Volunteer	LNLC, NINDS																								
COOPERATING UNITS (if any) Dept. of Neurosurgery, Children's Hospital National Medical Center, Washington, DC (Dr. Schiff)																										
LAB/BRANCH Laboratory of Neural Control																										
SECTION Section on Neural Mechanisms																										
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892																										
TOTAL STAFF YEARS: <div style="text-align: center;">3.5</div>	PROFESSIONAL: <div style="text-align: center;">2.7</div>	OTHER: <div style="text-align: center;">0.8</div>																								
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews																	
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<input type="checkbox"/> (a2) Interviews																										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The project is designed to provide information about the organization of neuronal systems in the mammalian spinal cord which ultimately controls the activity patterns of <u>motor units (motoneurons and the muscle fibers they innervate)</u>. Topics of interest include analysis of mechanisms of <u>synaptic transmission</u> in the spinal cord, of the <u>reflex pathways</u> within the spinal segment and control of information flow in them by input from <u>primary afferent and supraspinal descending systems</u>, and studies of the anatomy of spinal elements relevant to these physiological questions. Of particular current interest is the organization of synaptic input systems, both segmental and supraspinal, that project to particular motor pools and the interaction of these systems with the spinal mechanisms that generate rhythmic motoneuron output patterns underlying <u>locomotion</u>. Current work concerns the organization of <u>excitatory last-order interneurons</u> in the <u>cat</u> spinal cord, with particular reference to interneurons that transmit <u>short-latency excitation</u> from low-threshold <u>skin afferents</u> and from <u>reticulospinal systems</u> that travel in the medial longitudinal fasciculus (MLF). All of these interneuron groups are strongly influenced by the spinal <u>central pattern generator (CPG)</u> for locomotion. The differential patterns of CPG modulation indicate that separate systems of segmental interneurons, each with highly specific patterns of primary afferent and descending convergence, are present in the mammalian spinal cord. We have also studied the sources of variability of <u>motoneuron excitability</u> during monosynaptic reflexes, a subject with specific clinical applications.</p>																										

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01687-24 NLNC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Techniques for Making Connections with the Nervous and Musculoskeletal Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.J. Bak	Electronics Engineer	NLNC, NINDS
Others: R.E. Burke, M.D.	Chief	NLNC, NINDS
G.M. Dold	Engineering Technician	NLNC, NINDS
F.T. Hambrecht, M.D.	Health Scientist Administrator	DFN, NINDS
M.J. O'Donovan, M.B.Ch.B.	Section Chief	NLNC, NINDS
E.M. Schmidt, Ph.D.	Biological Engineer	NLNC, NINDS
W.J. Yee	Biological Engineer	NLNC, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.9

PROFESSIONAL:

0.3

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is intended to develop techniques and instrumentation for the acquisition and processing of neuroelectric signals from the central and peripheral nervous system in acute and chronic neurophysiological preparations. Because of this laboratory's continuing interest in sensorimotor neural activity during unrestrained movements, the project also includes development and fabrication of chronically implantable microelectrodes, mechanical transducers, catheters, and connectors. Arrays of chronically implantable "map-pin" electrodes are being adapted for long term human implantation as part of the ongoing study to determine the feasibility of a functional visual prosthesis for the blind.

Due to the laboratory's new interests in doing research on isolated, in-vitro preparations, a significant amount of work has been devoted to improving techniques associated with electrical recording, stimulation, and real time fluorescence microscopy in these preparations. Also included within this report is the development of computer programs of general utility for acquisition and analysis of neuroelectric and mechanical records, as well as of neuroanatomical material.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01688-24 LNLC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Cortical Mechanisms of Voluntary Motor Control

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.M. Schmidt, Ph.D.	Biological Engineer	LNLC, NINDS
Others:	M.J. Bak	Electronics Engineer	LNLC, NINDS
	A. Bragg	Summer Student	LNLC, NINDS
	D. Camesi	Biologist	LNLC, NINDS
	G.M. Dold	Engineering Technician	LNLC, NINDS
	W.J. Heetderks, M.D., Ph.D.	Health Scientist Administrator	DFN, NINDS
	J.S. McIntosh	Physiologist	LNLC, NINDS
	R. Dziedzic	Biological Aid	LNLC, NINDS

COOPERATING UNITS (if any)

University of Michigan (K. Wise), University of Utah (R. Norman)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Neural Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.2

PROFESSIONAL:

0.4

OTHER:

1.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
 ☐ (b) Human tissues
 ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to investigate the spatial distribution and functional properties of cortical neuron "colonies" in the primate motor cortex and supplementary motor area (SMA) that project to the spinal cord and are associated with individual muscles or closely related groups of muscles, as well as the activity of neurons in such colonies during defined voluntary motor behaviors.

Multicontact passive semiconductor electrodes have been successfully implanted in the arm area of the SMA of a primate that was trained to do a number of different wrist movement tasks. Cells seem to be better correlated with complex tasks than simple repetitive tasks. Recorded activity diminishes in amplitude after several weeks but can be restored with microstimulation through the electrode.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02079-19 LNLC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Models of Neurophysiological Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W.B. Marks, Ph.D.	Research Physiologist	LNLC, NINDS
Others:	R.E. Burke, M.D.	Chief	LNLC, NINDS
	M.J. O'Donovan, M.B.Ch.B., Ph.D.	Research Physiologist	LNLC, NINDS
	T.G. Smith, Ph.D.		LNLC, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Neural Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.2

PROFESSIONAL:

0.7

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As quantitative data become available for a particular form or function in the nervous system, it is advisable to attempt to assimilate the information into a comprehensive model of the underlying mechanisms and their interactions. This project consists in the development of such models, as well as the necessary analytical and mathematical techniques for their implementation and testing in several areas of experimental investigation carried out by LNLC members and in other laboratories.

Analytical dendrite algorithms

Our initial 16-parameter algorithm for modeling motoneuron dendrites is a Monte Carlo method; it produces a finite ensemble of random trees. We now have several methods for directly calculating the statistical properties of all possible dendrites using the parameters of the distributions of particular dendrite attributes. The simplest form is an analytical expression with 3 parameters for the distribution of branch diameter of each order. A more complete model with 6 parameters allows further calculation of the distributions of dendrite area and volume. Using the parameters of the full model we can calculate the expected number of branches of each diameter at each distance and the probabilities of a configuration of numbers of branches having each diameter, for computing the expected value of more complex attributes

Models of diffusion along axons

We have modeled passive diffusion along axons of dye injected into a ventral root of the chick embryo as it diffuses from the injection site to motoneurons in order to compare its time course with that of active transport. Dr. O'Donovan's measurements in this system agree in time course with the diffusion hypothesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02160-18 LNLC
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Intrinsic Properties of Motor Units		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: R.E. Burke, M.D. Chief, LNLC LNLC, NINDS Others: W.B. Marks, Ph.D. Research Physiologist LNLC, NINDS		
COOPERATING UNITS (if any) Dept. Anat., Karolinska Institutet, Stockholm, Sweden (B. Ulfhake); Dept. Physiol., Univ. North Carolina School Med., Chapel Hill, NC (R.E.W. Fyffe); Dept. Neurobiol., Institute of Life Sciences, Hebrew Univ., Jerusalem, Israel (I. Segev); Dept. Behavioral Neuroscience, Univ. of Pittsburgh (W. Cameron). Mathematical Research Branch, NIDDK (W. Rall)		
LAB/BRANCH Laboratory of Neural Control		
SECTION Section on Neural Mechanisms		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS: <div style="text-align: center;">1.5</div>	PROFESSIONAL: <div style="text-align: center;">0.8</div>	OTHER: <div style="text-align: center;">0.7</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project is designed to provide information about the populations of <u>motor units</u> that make up large limb muscle in mammals. The scope of work includes studies of the electrophysiological and morphological characteristics of <u>spinal cord motoneurons</u>, the <u>organization of synaptic inputs</u> to them, and the relationship of these central nervous system factors to the mechanical, histochemical and anatomic properties of the <u>muscle fibers</u> (termed "muscle units") innervated by the motoneurons. Current work on this project is largely focussed on <u>neuroanatomic studies</u> and <u>computer modeling</u> of individual, functionally-identified motoneurons, with emphasis on the fundamental features that control <u>dendritic morphology</u> and the influence of dendritic anatomy on the electrical properties of neurons and mechanisms of information processing in dendrites. We have devised a relatively simple stochastic model that is useful to isolate the key factors that control dendritic morphology. This approach is being used to compare the fundamental dendritic structure of several groups of cat motoneurons, as well as the morphologies of motoneuron dendrites in two groups of motoneurons during <u>postnatal development</u>. We are using the original data and computer-generated dendrites to explore the electrophysiological consequences of different dendritic structures. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 NS 02254-16 LNLC

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Repair of Injured Nervous Tissue with Foreign Grafts

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. A. Zalewski, M.D.	Section Chief	LNLC, NINDS
Others: N. A. Azzam, Ph.D.	Special Expert	LNLC, NINDS
R. N. Azzam	Histopathology Tech.	LNLC, NINDS
J.D. Ziemnowicz	NIH Special Volunteer	LNLC, NINDS

COOPERATING UNITS (if any)
CNS Disorders Research, The Upjohn Co., Kalamazoo, MI (L.R. Williams); Transplantation Laboratory, American Red Cross, Rockville, MD (G.M. Fahy)

LAB/BRANCH
Laboratory of Neural Control

SECTION
Section on Neuronal Regeneration

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.8	PROFESSIONAL: 2.0	OTHER: 1.8
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Three areas of research concerning peripheral nerve fiber regeneration were investigated. In one study in rats, denervated nerve stumps were sutured into opposite ends of 10-mm long impermeable silicone chambers. This surgical procedure was designed to determine whether nonneuronal cells from the ends of the nerve stumps (i.e., Schwann, endothelial and fibroblasts) would proliferate and migrate into the chamber and form a tissue cable in the absence of regenerating axons. Electron microscopy revealed that after four weeks cables formed in chambers, and that all nonneuronal cell types contributed to cable formation. However, cables formed only in chambers that were filled, at the time of surgery, with dialyzed plasma and not phosphate-buffered saline. Schwann cells in aneural cables were enclosed within basement membrane. Heretofore, it was believed that only axons induced basement membrane formation by Schwann cells. We now plan to anastomose a normal proximal nerve stump (i.e., one that contains axons) to aneurally formed cables to determine if these cables can support axonal regeneration through them. In another study, we attempted to cryopreserve nerves of rats for later use as nerve grafts. Peroneal nerves were successfully cryopreserved in a mixture of dimethyl sulfoxide and formamide (DF). Nerves in DF were stored in liquid nitrogen up to five weeks and survived subsequent transplantation as manifested by the presence in them of Schwann and perineurial cells and an endoneurial vasculature. Host axons were able to regenerate through 4-5 cm lengths of cryopreserved nerve isografts. However, when employed as allografts, cryopreserved nerves were rejected by normal, untreated rats but not by rats immunosuppressed with the drug cyclosporine. If human nerves can be cryopreserved and their rejection prevented by cyclosporine therapy, it should be possible to create human nerve banks from which grafts can be obtained to repair gaps in injured peripheral nerves. Pigeons were utilized to elucidate how the degenerating olfactory pathway prepares itself for olfactory axonal growth. The olfactory system is unique because after injury, olfactory neurons die and are replaced from stem cells that differentiate into new neurons. It is important to determine how the axons of these new neurons grow back into their target tissue in the central nervous system (CNS), the olfactory bulb. After unilateral olfactory nerve transection, olfactory axons disappeared and their supporting cells in the degenerating nerve stump arrange themselves into two concentric tube-like channels. The smaller, inner channel consisted of dramatically shrunken basement membrane which enclosed ensheathing (possibly Schwann) cells whereas the larger, outer channel was composed of fibroblast-like cells that lacked basement membranes. Further electron microscopic study should reveal which tube growing olfactory axons are utilized for growth into the CNS.

15 - LNLC/DIR

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02787-04 LNLN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Network Function in the Developing Spinal Cord of the Chick Embryo.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. J. O'Donovan, M.B.Ch.B.	Research Physiologist	LNLN, NINDS
Other:	M. Antal, M.D., Ph.D.	Visiting Associate	LNLN, NINDS
	S. Ho, Ph.D.	Visiting Fellow	LNLN, NINDS
	A. Lev-Tov, Ph.D.	Guest Researcher	LNLN, NINDS
	W.B. Marks, Ph.D.	Research Physiologist	LNLN, NINDS
	A. McClellan, Ph.D.	Guest Researcher	LNLN, NINDS
	D. McPherson, Ph.D.	Guest Researcher	LNLN, NINDS
	A.M. Ritter, Ph.D.	Guest Researcher	LNLN, NINDS
	A. Roberts, Ph.D.	Guest Researcher	LNLN, NINDS
	G. Sholomenko, Ph.D.	Visiting Fellow	LNLN, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

6.1

PROFESSIONAL:

3.7

OTHER:

2.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project is concerned with analyzing the development and function of spinal networks in the lumbosacral cord of the chick embryo. One focus of the study is on the synaptic organization of motoneurons. A second interest is in analyzing the cellular and network mechanisms responsible for the genesis of spontaneous motor activity. All experiments are performed on an isolated preparation of the spinal cord which is maintained *in vitro*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02788-04 LNLC									
PERIOD COVERED October 1, 1991 through September 30, 1992											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of Neuronal Shape*											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: C.L. Smith, Ph.D.</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">LNLC, NINDS</td> </tr> <tr> <td>Other: J. Drazba, Ph.D.</td> <td>IRTA Fellow</td> <td>LN, NINDS</td> </tr> <tr> <td>Sharon Walters</td> <td>Biologist</td> <td>LNLC, NINDS</td> </tr> </table>			PI: C.L. Smith, Ph.D.	Senior Staff Fellow	LNLC, NINDS	Other: J. Drazba, Ph.D.	IRTA Fellow	LN, NINDS	Sharon Walters	Biologist	LNLC, NINDS
PI: C.L. Smith, Ph.D.	Senior Staff Fellow	LNLC, NINDS									
Other: J. Drazba, Ph.D.	IRTA Fellow	LN, NINDS									
Sharon Walters	Biologist	LNLC, NINDS									
COOPERATING UNITS (if any) Albert Einstein University (S. Kahn), Case Western Reserve University (V. Lemmon)											
LAB/BRANCH Laboratory of Neural Control											
SECTION Section on Developmental Neurobiology											
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892											
TOTAL STAFF YEARS: <div style="text-align: center;">2.0</div>	PROFESSIONAL: <div style="text-align: center;">1.0</div>	OTHER: <div style="text-align: center;">1.0</div>									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"><input type="checkbox"/> (a) Human subjects</td> <td style="width: 33%;"><input type="checkbox"/> (b) Human tissues</td> <td style="width: 33%;"><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews		
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither									
<input type="checkbox"/> (a1) Minors											
<input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project uses structural methods to study neurons grown <i>in vitro</i> with the goal of understanding the molecular mechanisms involved in neurite outgrowth and pathfinding. Current work focuses on two problems: (1) <u>Cytoskeletal mechanisms and substrate interactions</u> involved in <u>initial neurite outgrowth</u>. The sequence of changes in cell shape and movements of the cytoskeleton accompanying neurite outgrowth from <u>peripheral ganglion</u> neurons grown <i>in vitro</i> was examined with <u>time-lapse videomicroscopy</u> and immunofluorescence labeling methods. Neurons initially form multiple filopodia which contain <u>actin microfilaments</u>, while <u>microtubules</u> and <u>neurofilaments</u> are restricted to the cell center. Formation of a neurite entails protrusion of cytoplasm containing microtubules and neurofilaments into a filopodium. Entry of microtubules does not appear to require polymerization of tubulin because microtubules enter filopodia during inhibition of tubulin polymerization. Transformation of a filopodium into a neurite is promoted by particular spatial patterns of substrate adhesion but does not require a specific cell adhesion molecule. (2) Influence of purified <u>cell adhesion molecules on growth cone</u> behavior. Growth cones of retinal neurons growing on substrates coated with either a purified cell adhesion molecule (laminin, L1, N-cadherin, or merosin) or polylysine was examined using time-lapse <u>laser scanning interference reflection microscopy (LS-IRM)</u> to show the spatial separation of the cell membrane from the substrate. There were marked differences between substrates in the overall extent of growth cone adhesion and in the variation in adhesion levels of individual membrane patches over time. Rate of growth cone migration did not bear a consistent relationship to either the overall level or temporal dynamics of substrate adhesion. </p> <p>*The title of this project has been changed from "Development of Primary Sensory Neurons" to reflect a broadening of its scope.</p>											

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02857-01 LNLC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Feasibility Study of an Intracortical Visual Prosthetic Device for the Blind

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.M. Schmidt, Ph.D.	Biol. Engineer	LNLC, NINDS
Others: M.J. Bak	Elect. Engineer	LNLC, NINDS
A. Bragg	Summer Student	LNLC, NINDS
G.M. Dold	Eng. Technician	LNLC, NINDS
A. Reina	Summer Student	LNLC, NINDS

COOPERATING UNITS (if any)

Fundamental Neurosciences Program, NINDS (W.J. Heetderks and F.T. Hambrecht); University of Michigan (K. Wise), University of Utah (R. Norman), Howard Hughes Fellow (P. Vallabhanath); Surgical Neurology Branch, NINDS (C.V. Kufta and D.K. O'Rourke)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Neural Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.2

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to evaluate the feasibility of a visual prosthesis for totally blind individuals by stimulating chronically implanted microelectrodes in the visual cortex. A 42-year-old woman who has been blind for 22 years was implanted with an array of 38 electrodes in the visual cortex. Stimulation of individual electrodes produced sensations of light called phosphenes. Phosphenes were produced with 34 of the 38 electrodes with currents that were 100 to 1000 times lower than had been reported for surface stimulation of the visual cortex. Additional blind patients have to be tested before we will know if intracortical microstimulation (ICMS) of the visual cortex is a feasible technique for producing a visual prosthesis. However, all the tests performed to date indicate that ICMS may be a feasible technique.

ANNUAL REPORT

October 1, 1991 through September 30, 1992

Laboratory of Neurobiology
Basic Neurosciences Program, DIR
National Institute of Neurological
Disorders and Stroke

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ANNUAL REPORT
October 1, 1991 through September 30, 1992
Laboratory of Neurobiology, DIR
Basic Neurosciences Program
National Institute of Neurological Disorders and Stroke
Thomas S. Reese, M.D., Chief

The Laboratory of Neurobiology (LN) has three Sections. The Section on Structural Cell Biology uses modern structural and biochemical techniques to investigate basic cell biology problems germane to an understanding of the function of nerve cells. The Section on Analytical Cell Biology applies a variety of quantitative physical techniques to similar issues, and the Section on Brain Structural Plasticity works on problems of fundamental and clinical importance in the mammalian CNS, especially those related to regeneration and response to injury. Current emphasis of the Sections on Structural Cell Biology and Analytical Cell Biology is on the mechanisms of axoplasmic transport, the structure of macromolecular assemblies that support axonal transport, axonal growth, calcium regulation in nerve and muscle, and synaptic function. The Section on Analytical Cell Biology is also committed to developing new quantitative approaches in advanced microscopy and to working on the organization and assembly of specialized membranes in glia and muscle. The Section on Structural Plasticity is currently investigating factors which promote establishment and disruption of blood-brain barrier function and neural connections in tissues implanted in the brain.

It is the Section on Structural Cell Biology that discovered the molecular basis of the directed organelle movements underlying fast axoplasmic transport. The protein motor for fast anterograde transport belongs to a new class of motility proteins named kinesins. Kinesins also occur in many non-neuronal cells, and appear to be a general and fundamental effector of intracellular movement. The goal of current work in this Section is to understand how kinesins and other protein motors carry out neural functions, such as fast axonal transport, organization of endoplasmic reticulum, and the cytoplasmic movements associated with slow axonal transport. Video microscopy, image processing, biochemistry, and advanced electronmicroscopy are applied to living axons, *in vitro* models, and reconstituted systems to determine the molecular events that underlie various motor functions.

A powerful new instrument, the field-emission scanning transmission electron microscope (FE-STEM), has been used on frozen specimens prepared by a new structural technique recently developed in LN to visualize directly motor proteins in their native configurations. Low-dose, dark-field images from this microscope allow, for the first time, kinesin and other motors to be visualized directly on the surfaces of microtubules and organelles. The FE-STEM was used in a collaborative study within LN and with the Biomedical Engineering & Instrumentation Program of NIH (where the FE-STEM is housed) to determine, at 2 nm resolution, the shapes and mass distributions of purified squid brain kinesin molecules—both free and bound to microtubules. Individual kinesin molecules appeared as asymmetric dumbbell-shaped structures (47-51 nm long, MW = 374 ± 14 kD), with a large head, stalk, and smaller globular foot. This structure is quantitatively consistent with models derived from biochemistry and molecular biology. Maps of kinesin bound to purified, taxol-stabilized bovine and squid microtubules by a nonhydrolysable analog of ATP, along with images of thin-film replicas prepared by a new technique

developed in LN this year, revealed that *both* ends of kinesin bind to microtubules. Thus, individual kinesin molecules can act as the bridges to bundle microtubules. These results are the first direct evidence for cross-bridging of microtubules by single kinesins and suggest that the cytoplasmic pool of kinesin might translocate microtubules. Thus, microtubule sliding along other microtubules, as reported by other laboratories, could be of physiologic significance, possibly in cytoplasmic movements such as slow axonal transport.

Reexamination of the effects of axoplasmic supernatant on organelle movements *in vitro* yielded the surprising finding that the cytoplasmic pool of kinesin does not appear to be directly involved in promoting organelle movements. Organelles which have exogenous proteins stripped from their surface by high-salt treatments show vigorous movement in the absence of supernatant. Addition of supernatant produces approximately 50% more movements, but these disappear when dynein in the supernatant is inhibited, so the kinesin in the supernatant has little or no effect. Thus, the kinesin-like motor appears to be tightly bound to the organelles rather than in equilibrium with the free cytoplasmic pool. Preliminary immunoblots of proteins eluted from organelles with detergent show that a kinesin-like protein is tightly bound to their surfaces; this protein is now being characterized by a collaboration with workers outside LN. This problem is also being approached structurally within LN by colloidal gold immunocytochemistry of kinesin-organelle-microtubule complexes. The most recent advance is to be able to separate, by affinity purification, the anterograde, kinesin-driven organelles from the retrograde, dynein-driven organelles. This result appears to be an important step in understanding how the direction of transport is determined as the retrograde organelles are moved along microtubules by dynein, but not by kinesin.

A remaining and important question is how kinesin is organized in axonal cytoplasm and on organelle and microtubule substrates. Since squid axoplasm contains a large pool of readily solubilized kinesin, immunocytochemical methods depending on cross-linking fixatives can give spurious results if this protein attaches to organelles during fixation. A considerable effort to develop a method for localizing soluble proteins has culminated in a method which uses post-embedding immunocytochemistry after freeze-substitution and cryoembedding. Applied to squid axoplasm, this method shows (in distinction to the published results with aldehyde fixatives) that most of the stainable kinesin is in the cytoplasmic pool, and that there is a separate and distinctly more concentrated pool on organelle surfaces. The fact that there is a large cytoplasmic pool of kinesin and that this pool apparently does not contribute to organelle movement suggests that the cytoplasmic kinesin might have other functions, such as the interactions with microtubules (see above) or endoplasmic reticulum (see below).

A functional kinesin molecule has four subunits, two heavy and two light chains; it is the light chains, in conjunction with the C-terminal ends of the heavy chains, which appear to be responsible for binding kinesin to organelles, ER, or other substrates that are transported by kinesin. It therefore becomes of interest to clone and characterize kinesin light chains in order to develop methods to explore factors regulating the functions of motor proteins on organelle surfaces. Since recent evidence suggests that there is a family of kinesin heavy chain proteins, there may also be a family of light chains. Thus, it is possible that different combinations of heavy and light chains could have a role in binding to different organelles to regulate motor function. The characterization of kinesin light chains in the squid is being undertaken to define the diversity of light chains in the nervous system

and ultimately to determine function and specificity with regard to intracellular transport, particularly axonal transport in neurons, of membrane-bound organelles. So far, it appears from cDNA sequencing that there is a family of light chains suggesting that they may have disparate though, perhaps, overlapping functions within the nervous system.

Another project using molecular techniques addresses the relationship of the molecular organization of cytoplasm to the functional polarity of the excitable cells. There has recently been increased interest in the mechanism of cell polarization during development. For example, axons and dendrites differ in their protein and organelle content; at the neuromuscular junction the muscle cell membrane is highly enriched in a specific subset of proteins. The formation and maintenance of such specialized domains can now be studied directly by using new approaches that take advantage of the advances in molecular biology. The specific localization of proteins and mRNAs in muscle cells are being addressed. A tissue culture system of hybrid myotubes is used to follow the distribution of transfected proteins and mRNAs produced from a single nucleus in a multinucleated myotube. One of the interesting results of this work, done before beginning the project in LN, was to show that mRNAs were distributed independently of the proteins they encode. This experimental system will be used to dissect the factors controlling mRNA, protein, and organelle localization in muscle cells. The combination of new molecular and cell biology techniques under development, combined with the structural techniques that have been pioneered in LN, will be a fruitful way to approach the question of structural polarity in excitable cells. A new program started last year addresses the specific localization of proteins, mRNAs and subcellular organelles in muscle and nerve cells. New tools have been developed: a low-density culture system of rat hippocampal neurons has been established in the Laboratory and several techniques have been tested to obtain expression of foreign in these neurons. Transfection of plasmid DNA by the Transfectam technique has given good results with the reporter gene for β -galactosidase.

Other new projects in molecular biology explore, at the molecular level, the roles a number of regulatory molecules, including A kinases, the modulatory neuropeptides buccalin and myomodulin and rab 3 proteins, play in neural function. Two different invertebrate systems are currently being studied. Synaptic plasticity is being studied as a consequence of changes in the distribution and substrate specificity of A kinase catalytic subunits in response to the application of extracellular stimuli to sensory neural clusters in the sea hare *Aplysia californica*. Secondly, the peptide families of buccalin and myomodulin in an attempt to gain further insights as to how peptide families collectively contribute to pre- or postsynaptic neuromodulation. The molecular characterization of peptide receptors through which signal transduction cascades are activated to affect modulation, for example G protein-coupled receptors, is also being actively pursued. Similarly, cloning of the small GTP-binding proteins is being pursued to determine the role rab 3a, in particular, plays in synaptic transmission.

The bacterial flagellar motor found in common bacteria is being used as a model system for cytoplasmic motors which has the advantage that the molecular biology is well worked out making close correlation with the structure feasible. These protein motors, unlike kinesin, are driven by ionic gradients, but like the axonal transport system they can switch the direction of translocation. A newly discovered structural component of the flagellar motor has been analyzed by both freeze-substitution and freeze-etch electron microscopy, showing that it is in an ideal position to both power flagellar rotation and to

contain the switch for changing its direction. Recently, the thin-film replica method has been successfully applied to characterize better this new structural component. Structural methods are now serving as an initial assay for a molecular genetic analysis of the putative switching component.

The structure and function of the endoplasmic reticulum (ER) in neurons and glia are being investigated using newly developed techniques which make it possible to investigate the dynamic properties of the ER in living cells by video and laser scanning confocal microscopy. New techniques have been developed using the sea urchin egg as a model system. The sea urchin egg has the advantages of being readily available, having a prominent ER, and being easy to microinject. A novel technique for specifically staining ER in living cells shows that the ER undergoes movements in the absence of microtubules, that a striking change in its organization occurs at the time of calcium release, and that the appearance of microtubules has a profound effect on its organization. By using this technique on fixed cells, the continuity of ER membranes can be determined. These techniques have recently been successfully adapted so they can be applied to neurons and muscle cells. The first results indicate that the ER is continuous throughout the Purkinje cell dendrites. Future work will focus on understanding the organization and dynamics of the ER in cultured neurons. A second initiative uses calcium-sensitive fluorescent indicators to investigate calcium regulation by the ER. In a cell-free preparation derived from sea urchin eggs, it was demonstrated that the ER is the source of InsP_3 -induced calcium release. Cytosolic calcium is being investigated with the laser scanning confocal microscope using a novel improvement in fluorescent calcium indicators. Application of these sensitive calcium indicators to the question of the distribution of calcium entering axons is now being investigated.

Structural changes in postsynaptic densities may underlie long-term modifications of synaptic activity. The aim of this project is to study the molecular organization of the postsynaptic densities and to explore the potential mechanisms for their modification in response to calcium and other intracellular messengers. Results obtained during the past year using postsynaptic density preparations from cerebral cortex indicate that the calcium calmodulin-dependent protein kinase, which is the most abundant protein in the densities is a strong candidate for mediating such structural modifications. We show that the densities contain an endogenous phosphatase of type 1 that actively dephosphorylated the calcium calmodulin-dependent protein kinase. When this phosphatase is effectively inhibited, almost all of the postsynaptic-associated kinase pool becomes phosphorylated. Modification of the postsynaptic density structure in response to elevated levels of calcium may also occur through selective degradation of component proteins by calpain, a calcium-activated protease. Preliminary studies that show different rates of degradation of different component proteins upon treatment of the postsynaptic density preparation with exogenous calpain, support this hypothesis. In the coming year, we shall continue studies on the biochemical and morphologic characterization of postsynaptic densities. To study the consequences of autophosphorylation of the kinase, we shall take advantage of our findings on the use of the phosphatase inhibitor, Microcystin-LR, to obtain a postsynaptic density preparation with a fully phosphorylated pool of calcium calmodulin-dependent protein kinase. The consequences of autophosphorylation and of limited degradation of components by calpain on morphology, protein-protein interactions, and other biochemical properties of the postsynaptic densities will be investigated.

Cytoplasmic polarity is expressed in the interactions of the cell with its exterior environment by means of adhesion molecules. A new initiative is aimed at characterizing differences in the adhesive patterns and associated growth characteristics of neuronal growth cones on defined substrates. Neurite formation by embryonic retinal ganglion cell neurons is observed using a technique (time-lapse laser scanning interference reflection microscopy) to show local distances of the cell membrane from substrates composed of purified, biologically relevant cell and substrate adhesion molecules. Members of the three major classes of adhesion molecules have been tested - the calcium-dependent, the calcium-independent, and the immunoglobulin superfamily of molecules. The overall degree to which growth cones bind to different molecular substrates varies widely, but a trend can be seen between reduced adhesivity and increased growth cone motility, suggesting that increasing levels of adhesivity may not be the basis of growth promotion as previously suggested. Analysis of the dynamic changes in adhesion patterns also shows wide variation among substrates. Since growth cones *in vivo* may encounter many molecules simultaneously, it seems reasonable to suggest that they must be able to integrate these multiple signals into a response that is suitable for directing them to their appropriate target. There may be a critical level and, perhaps, pattern of adhesion of a growth cone to its substrate that is necessary for neurites to form and move forward. Experiments will continue to analyze adhesive interactions to molecules in combination and in patterned arrays the role of cytoskeletal interactions with adhesion molecules on the cell surface is being explored as well.

The Section on Analytical Cell Biology, now in its second year, studies problems of membrane organization and calcium regulation in excitable cells using modern low-temperature, microscopic and analytic techniques. The projects in the Section are organized around a set of related themes: 1) calcium regulation by membranes and organelles in nerve and muscle; 2) formation and function of specialized (often calcium-regulating) membranes such as synapses between CNS neurons, triad junctions of skeletal muscle and myelin sheaths of Schwann cells and oligodendrocytes; and 3) structure of macromolecular assemblies that underlie intracellular transport. In addition, the Section is committed to the development of new technologies in biologic light and electron microscopy.

An example of such a technologic development is the unique field-emission scanning transmission electron microscope (FE-STEM) modified in collaboration with R.D. Leapman in the Biomedical Engineering & Instrumentation Program at NIH for low-temperature, high-resolution biological work. Last year, we demonstrated near-single atom sensitivity for calcium and phosphorus analysis of macromolecules by parallel electron energy loss spectroscopy (PEELS). This technique, in combination with the established low-dose dark-field mass mapping capabilities of the FE-STEM, means that low-dose structural imaging followed by high-dose elemental analysis of the same molecule is a practical approach to characterize coordinated elemental and shape changes in macromolecules. Now, an extension of this approach has been shown to work for tissue cryosections. Thus, PEELS "spectrum imaging" and dark-field mass mapping have been used to determine the distribution of calcium and proteins, respectively, within the organelles of Purkinje cell dendrites. This work confirms the conclusion, previously drawn from X-ray microanalysis and described in more detail below, that the endoplasmic reticulum (ER) is a heterogeneous population of organelles which are nevertheless the major calcium storage organelles in these dendrites.

Darkfield analysis is also useful to determine the water content of individual organelles, which is an important physiologic parameter that changes during neuronal activity. Since the darkfield approach is indirect, however, a new and different method to map the water content of subcellular organelles directly, at high resolution and without electron beam damage, has been developed. This approach depends on analysis of the low-loss region of PEELS spectra, which can be obtained as a high-resolution map of a frozen-hydrated cryosection, in order to extract the water fraction. The intensity of the low-loss signal allows the acquisition of these maps at low dose and therefore without electron beam damage, so that the specimen is still suitable for further imaging and analysis as a freeze-dried specimen.

Another analytic capability of the FE-STEM which has proven beneficial is high-sensitivity, high-resolution, energy-dispersive X-ray microanalysis. X-ray microanalysis is a technique that can localize and measure total calcium and other elements within subcellular compartments of cells in ultrathin cryosections of directly frozen tissues. Recently, we reported major improvements in the quality of such cryosections—improvements that in combination with the FE-STEM permitted new experiments to characterize calcium sequestration during synaptic activity in parallel fiber/Purkinje cell synapses of the cerebellar cortex. Previous studies had shown that the highest concentrations of calcium were contained within the ER of both dendritic shafts and spines. Moreover, the amounts of sequestered calcium depended on synaptic activity, and were different for dendrites *vs.* spines. The question of how these anatomically different cisterns accumulate and release calcium in response to postsynaptic transmitter binding has become increasingly interesting, as recent immunolabeling and physiologic experiments have demonstrated the presence of a variety of plasma membrane calcium conductances and intracellular calcium release channels.

New results, based on X-ray analyses of ER calcium paired with the surrounding cytoplasm, reveal that dendritic spines actually cycle between three distinct physiologic states: (1) high calcium in the ER and low in the cytoplasm; (2) low calcium in the ER and high in the cytoplasm, with the same total amount as in case (1); and (3) high calcium in both ER and cytoplasm. These states likely reflect unactivated, recently activated, and long-term activated synapses, respectively. These data provide direct evidence for, as well as yielding quantitative data about, the operation of intracellular calcium stores within spines; in the case of Purkinje cells, this indicates the presence and importance of inositol trisphosphate (IP_3)-sensitive calcium release channels within the membranes of spine ER. The high-calcium ER/high-calcium cytoplasm spine represents an attractive candidate for a structure which has undergone activity-dependent, long-term changes in synaptic efficacy. These experiments have also defined the more complicated, multimodal calcium distribution within ER of dendritic shafts. As one type of dendritic ER is similar to that found in spines, this will likely prove to be an IP_3 -sensitive storage organelle.

Changes in the cytoplasmic calcium levels serve as the immediate signal for muscle contraction. During excitation-contraction coupling, depolarization of the transverse (T) tubule membrane is transduced into the rapid release of calcium from an intracellular store, namely, the sarcoplasmic reticulum (SR). The structure where this occurs—the triad—requires fast interactions between, and therefore the proximity of, the voltage sensor and the internal calcium release channel. By visualizing (with dyes and antibodies) the specific membranes, *viz.*, T-tubules and SR, associated with specific membrane proteins, the

development and molecular composition of the triad can be determined. Recently, we studied the organization of the dihydropyridine-sensitive calcium channel (which is thought to be the voltage sensor mediating excitation-contraction coupling) and the calcium release channel of the SR (also called the ryanodine receptor). Both membrane channels are expressed early in development and appear clustered in receptor-rich domains of the T-tubules and SR, respectively; these clusters presumably represent developing triads. In myotubes of the dysgenic mouse mutant (*mdg*), the lack of the α_1 subunit of the dihydropyridine receptor results in failure of the α_2 subunit to aggregate and to be retained in the T-tubule membrane. This implies that specific interactions between the α_1 subunits are required for targeting and assembly of the receptor complex in the junctional T-tubules. Expression of the ryanodine receptor is reduced in dysgenic myotubes and it frequently fails to form clusters. Despite lack of the dihydropyridine receptor in dysgenic myotubes, however, the ryanodine receptor can attain a high degree of differentiation indistinguishable from normal myotubes. Thus, the molecular differentiation of the junctional membrane of the SR are independent from interactions with the dihydropyridine receptor. The expression of α_1 and the normal distribution patterns of both dihydropyridine receptor subunits as well as that of the ryanodine receptor can be locally restored by the fusion of dysgenic myotubes with normal cells, resulting in the rescue of excitation-contraction coupling.

Immunocytochemical studies on the developing excitation-contraction coupling apparatus were paralleled by recordings of free cytoplasmic calcium using microfluorometry and imaging of fluorescent calcium indicators in normal and dysgenic myotubes. In addition to action potential-induced calcium transients, spontaneous and caffeine-induced transients were found in developing myotubes. These transients were not inhibited in the dysgenic mutant, indicating that they represent calcium release from the SR not under the control of the dihydropyridine receptor. Parallel investigation of the molecular assembly and properties of the excitation-contraction coupling apparatus during myogenesis promises further insight into the molecular interactions involved in the formation and function of this complex signal transduction system.

Another project which addresses questions of cell polarity and intracellular transport concerns myelin formation and maintenance. We have previously defined several distinct pathways for synthesis, transport and assembly of different myelin-specific proteins in oligodendrocytes and Schwann cells; at least some of these pathways rely on directed transport along microtubules. We have already described the organization of the microtubule network in myelinating Schwann cells, including its asymmetric disposition and bidirectional polarity. New immunocytochemical studies on Schwann cells in colchicine-treated nerves show that the major myelin proteins P_0 and MAG are sorted into separate carrier vesicles in the trans-Golgi network, i.e., at the Golgi/microtubule interface. Disruption of the microtubule network causes both types of vesicles to accumulate in the perinuclear region, but the P_0 -rich fraction can still fuse into myelin-like membranes. Transport of myelin proteins can also be disrupted at more distal sites, in cytoplasmic channels of the internode, by cytoskeletal rearrangements that include intermediate filaments and actin as well as microtubules. Thus, it would seem that Schwann cells invoke a rich variety of mechanisms for sorting and targeting of specific proteins to their appropriate membrane domains.

The three objectives of the Section on Brain Structure Plasticity are (A) to see whether axon regrowth through a compression lesion to the spinal cord is promoted by repeated injections of exogenous, activated macrophages and monocytes; (B) to identify the conditions whereby PC12 cells, differentiated by the ras oncogene, survive when transplanted to brain; and (C) to identify the determinants of a blood vessel's phenotype.

(A) Exogenous Macrophages and Spinal Cord Repair. The goal is to deliver exogenous, secretory macrophages (MØ) to the site of a spinal cord injury so as to provide cytokines and growth factors conducive to axon regeneration. Although CNS axons can regenerate, spinal cord injury in mammals typically leads to ischemic necrosis and the formation of cysts devoid of cellular bridges that would act as a scaffolding for regrowing axons. The progressive cavitation at the injury site has been attributed to the accumulation of endogenous, debris-laden MØ in their phagocytic, catabolic mode. Such phagocytic MØ release tumor necrosis factor, prostaglandins, free oxygen radicals, and proteases which damage or destroy cells. However, in their secretory, anabolic stage, MØ release vasogenic cytokines such as transforming growth factor- β and interleukin-1 and mitogenic growth factors. These agents promote tissue repair. If secretory MØ can be brought repeatedly to the damaged spinal cord, in sufficient numbers and at critical times during the progression of the lesion, they may promote the regrowth of axons across the defect. An activating agent such as bacterial lipopolysaccharide, known to enhance repair of CNS tissue, also primes MØ for their secretory phase.

The method is to compress, epidurally, the T-5 segment of inbred rats' spinal cords with forceps, a simple method known to be as reproducible as more elaborate methods involving force-measuring devices. Over the course of the 3 weeks following the trauma, MØ, taken from the peritoneal of donor rats and activated by lipopolysaccharide, are repeatedly injected intraperitoneally as boli of $\sim 2 \times 10^4$ cells at various times after the trauma: (i) 7 and 19 days; (ii) 7, 10, 14 and 18 days; (iii) 1, 4, 8, 12, 16 and 20 days. At 1 to 2 days after the last infusion of cells, the rats are fixed with aldehyde and frozen sections of the cord are examined by fluorescence and bright field microscopy.

The results of these preliminary experiments indicate that, like the injury of untreated rats, there is extensive cavitation of the spinal cord around the site of the lesion. A small but appreciable number of exogenous MØ reached the lesion. Those MØ that are small or large in size, oval, with diffuse fluorescence are, accordingly, regarded as secretory; they are situated primarily in regions that are cellularly dense. Large, irregularly shaped MØ with granular, i.e., lysosomal, fluorescence are, because of these features, probably phagocytic; they reside chiefly in areas of the lesion that are cellularly less dense. This pattern of tissue response and cellular infiltrate was the same in experimental groups 1 and 2. However, the third group, which received the first infusion of cells only 24 hr after the lesion and five additional infusions over a three-week period, had less cavitation and more areas of densely cellular tissue.

These initial results signify that: (a) small but appreciable number of exogenous, activated and labeled MØ can migrate from the peritoneal cavity to a spinal cord lesion; and (b) the secretory phase of the MØ, the timing of the infusions, number of infusions and the number of cells in the infusions are factors that likely affect the outcome of the treatment. Their order of importance has yet to be established.

The next steps are to: (i) to define the conditions that bring MØ and monocytes into their secretory phase; because monocytes are more readily activated by lipopolysaccharide and their secretory stage more accurately estimated, they, in addition to MØ, will be used; (ii) increase the number of activated cells in each infusion; (iii) inject the activated

monocytes and MØ intravascularly in some of the rats; and (iv) carry out planimetric morphometry of the cystic cavities in control and treated groups.

(B) PC12 Cell Survival. We continue our efforts to determine why PC12 cells, neuronally differentiated by the ras oncogene, survive when grafted to mature brain whereas naive PC12 cells, transplanted to the same brain, disappear within 1-2 weeks. The disappearance may be due to immunologic rejection because the naive PC12 cells survive to form tumors when we graft them to immunologically incompetent, athymic rats. The possibility that the cells' major histocompatibility complexes (MHC) have been altered by the oncogene were to be assayed by fluorescence-activated cell sorting after it was established, by visual inspection of coded slides, that the immunohistochemical differences between naive, NGF and ras groups of cells. Differences in immunostaining have been equivocal and further evaluation requires reproducible and optimal conditions for incubation with the antibodies before the cell sorter can be used.

Our previous finding that the NILE or L-1 cell adhesion molecule is expressed in NGF-treated PC12 cells but is diminished in ras-infected cells, suggests that the persistence of PC12 cells *in vivo* may have to do with cell-cell and cell-substrate attachment. One such cell surface molecule that may be relevant is sialic acid. As a first approach, the binding of the lectin, wheat germ agglutinin (WGA), is being measured. Although this particular lectin is not specific for sialic acid but also binds to N-acetylglucosamine, WGA has been chosen because (a) the NGF receptor is a glycoconjugate containing both sialic acid and N-acetylglucosamine; (b) WGA binds to both, but the two monosaccharides can be distinguished by using chemical derivatives of the lectin; and (c) it is known that WGA alters the properties of the NGF receptor and hence alters responses to this polypeptide growth factor. We have recently shown that NGF-treated PC12 cells and ras-PC12 cells express markedly more receptors for WGA than do naive cells; ras-PC12 cells bind about 98% more ¹²⁵I-WGA than do naive PC12 cells, while NGF-treated PC12 cells bind about 79% more. The significance of these results is that ras-PC12 cells have more sialic acid and, accordingly, a greater negative charge on their surface. We have confirmed that WGA blocks neurite regeneration reversibly and dose-dependently in NGF-primed PC12 cells. We have now gone on to show that this inhibition does not take place in ras-primed cells, a finding which signifies that the NGF receptor is not directly involved in neurite regeneration in ras-differentiated PC12 cells. The inhibition is being verified on partially cloned cells that are not susceptible to the toxic effects of WGA by testing their response to NGF and to ras oncogene. The significance of the differences in sialic acid is that changes in the type and amount of cell surface molecules may be involved in the survival or disappearance of PC12 cells in the brain and that the analysis by reflection interference microscopy, of adhesion, neurite extension and, possibly the movement, of naive, NGF- and ras-treated PC12 cells on various substrates *in vitro* is warranted.

In order to better define how NGF binding or ras-oncogene uptake by PC12 cells brings about neurite extension and the other responses that we had previously noted, the turnover of inositol phospholipid is being examined. This turnover is associated with an increase in intracellular Ca²⁺ ions which, in turn, modulate subsequent physiologic processes. Information from various extracellular signals, e.g., polypeptides such as NGF, are transmitted from the cell surface to the cell's interior through two main routes: Ca²⁺ mobilization and protein kinase activation. We have thus been examining, collaboratively, diacylglycerol with a sensitive radioenzyme assay on cultures of synchronized cells. Diacylglycerol levels increase five-to-six fold in both NGF and ras-treated PC12 cells

compared with naive cells, by 48 hr after treatment. We now must verify these experiments with a different assay, such as TLC, and in nonsynchronized cells as well.

The cell cultures used in both the *in vivo* and *in vitro* studies have been, initially, slightly contaminated by the inclusion of naive PC12 cells that have either not been infected with the ras oncogene or have not responded to its p-21, regulatory protein product. These few cells continue to divide and in long-term cultures may affect the results of various assays and *in vivo* results. So as to avoid the inclusion of naive PC12 cells in cultures of ras-PC12 cells, we have now been infecting the initial cultures with a murine sarcoma retrovirus that contains both the K-ras oncogene and the Neo-R gene that imparts resistance to the neomycin killing of cells. Those mitotic naive cells that do not incorporate ras are also deprived of the Neo-R gene and can, consequently, be removed by treating the cultures with neomycin. The results obtained from these purer populations of ras-PC12 cells should be interpretable with greater certainty.

(C) Determinants of Blood Vessel Phenotype. These experiments are designed to test the hypothesis that the phenotype of a blood vessel supplying a tissue is determined by that tissue rather than by the source of the vessel. It is known that permeable, sprouting mesenchymal blood vessels, upon entering a piece of developing, avascular brain, take on characteristics of the blood-brain barrier rather than retaining the permeable features of the source, mesenchymal vessels. The hypothesis derived from these observations is that the target tissue rather than the source tissue determines the type of vessel within the target. We have evidence to suggest that the hypothesis is not valid for mature tissue. When we place autografts of skeletal muscle on or within the choroid plexus of the IV ventricle in adult rats, the grafts become vascularized by fenestrated vessels, like those of the choroid plexus rather than the continuous type of vessel characteristic of muscle. The grafts' fenestrated vessels are actually surrounded by muscle cells with few or no intervening processes; endothelium and muscle cells are as close as 0.08 to 0.5 μm . The vessels are thus embedded in the target's milieu, yet they retain the fenestrae of the source vessels.

The significance of this finding is that either the mature, fenestrated vessels are no longer responsive to inducing factors that might be secreted by the muscle cells that would be expected to induce the vessels to become the muscle type or that the grafts of mature muscle no longer make and release the factor. It is likely that the hypothesis is valid only when both the source vessels and target tissue are immature. Preliminary experiments, in which the skeletal muscle graft is fetal rather than mature, have yielded only a few fetal muscle fibers and the type of vessels supplying them is being evaluated. The critical experiment would be to co-graft mammalian fetal muscle together with fetal choroid plexus. One system that could be used for the cograft is the adult rat's IV ventricle, which is able to support both central and peripheral neural and nonneural tissue.

An intriguing but unexplainable finding was that this result was obtained in 4 of 5 ganglionectomized rats but only a very few fenestrated vessels were found in 1 of 4 normal rats that received the autografts. This result suggests that the absence of their adrenergic innervation might affect the sprouting of fenestrated, choroidal vessels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-01442-25 LN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Permeability of Cellular Layers in the Vertebrate Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas S. Reese, M.D.	Chief	LN, NINDS
Others:	Marcelo Hernandez, M.D.	Exchange Scientist	LN, NINDS
	Bechara Kachar, M.D.	Visiting Scientist	LNO, NIDCD

COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543. R.C. Wagner, University of Delaware, Newark, DE.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892. Marine Biological Laboratory, Woods Hole, MA

TOTAL STAFF YEARS:	1.0	PROFESSIONAL:	0.5	OTHER:	0.5
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

How tight junctions might prevent small charged solutes from entering the brain (across the blood-brain barrier) is made clear by our new model of tight junction structure based on a lipidic backbone. Tight junctions in invertebrates also appear to have lipidic backbones though our most recent observations suggest that periodic structures, presumably proteins are intercalated into these backbones. This new work has been published. New methods depending on atomic force microscopy are under development to study junction structure in more detail. Otherwise this project is in abeyance.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-01881-22 LN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Basis of Synaptic Transmission

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jorge E. Moreira, Ph.D.	Visiting Scientist	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Gadi Benschalom, Ph.D.	Visiting Scientist	LN, NINDS

COOPERATING UNITS (if any)

R. Llinas, P.M. Reuss. Department of Physiology and Biophysics, New York University Medical Center, New York, NY 10016.

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SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892. Marine Biological Laboratory, Woods Hole, MA

TOTAL STAFFYEARS:

1.4

PROFESSIONAL:

1.0

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project deploys a range of structural techniques to examine normal synaptic structure. These approaches have in common their dependence on rapid freezing as well as direct visualization of living brain and both these approaches require direct access to fully viable living brain. Since such preparations are not available, the project is now engaged in exploring various live brain preparations. The most currently active part of this project has been aimed at developing a whole brain preparation that is maintained indefinitely *in vitro* by vascular perfusion as well as superfusion with artificial CSF. This approach has currently been very successful in maintaining normal structure of the hippocampal and cerebellar cortices for up to 90 minutes of perfusion. Another approach, that has been tried extensively in LN is to develop organotypic brain slice cultures. A comprehensive effort to develop methods for making and maintaining organotypic brain cultures continues (see also Project #Z01 NS 02610-08 LN). It has proven consistently difficult to produce large expanses of mature brain but this preparation is currently being reevaluated. Parts of this project having to do with neuronal development and with synaptic biochemistry have now been established as independent projects (see #s Z01 NS 02871-01 LN and Z01 NS 02872-01 LN).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02551-11 LN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Cytoplasmic Motors*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas S. Reese, M.D.	Chief	LN, NINDS
Others:	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	S. Brian Andrews, Ph.D.	Section Chief	LN, NINDS
	Mark Terasaki, Ph.D.	Senior Staff Fellow	LN, NINDS
	Shahid Khan, Ph.D.	Guest Researcher	LN, NINDS

COOPERATING UNITS (if any)

R.D. Leapman, BEIP, NCCR, NIH, Bethesda, MD. B.J. Schnapp, Department of Cellular and Molecular Physiology, Harvard Medical School, Boston, MA. T. Slater, A. Fein, Department of Physiology, Northwestern Univ Medical School, Chicago, IL.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892. Marine Biological Laboratory, Woods Hole, MA

TOTAL STAFFYEARS:

2.4

PROFESSIONAL:

2.4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to understand how motors which power fast axonal transport promote movement. An important current question is how kinesin or dynein is organized on the organelle surface and microtubule substrate. A scanning transmission electron microscope (STEM) (as described in Project #Z01 NS 02610-09 LN), has allowed kinesin to be visualized directly on the surfaces of microtubules and organelles. Maps of kinesin bound to purified, taxol-stabilized bovine microtubules provided the first direct evidence for cross-bridging of microtubules by single kinesins which suggests that kinesins in cells might also translocate microtubules and therefore have some role in slow as well as fast axonal transport. These observations are now being extended to organelles, where we have recently been able to separate anterograde, kinesin-powered organelles, from retrograde, dynein-powered organelles, and to endoplasmic reticulum (ER) where we have shown incidentally that the ER makes one continuous system throughout the Purkinje neuron. The dynamic properties of other biological motors are being studied for comparison with the axonal transport motors; the bacterial flagellar motor in E. coli has also been shown to depend on interactions of the flagellar structure with membrane proteins although these motors are driven by rather than controlled by ionic gradients. This motor system, like the axonal motor system, can also switch direction of translocation. A newly discovered structural component of the flagellar motor has led us to propose a novel structural model. Molecular genetic analysis of the new structural components is expected to lead to an understanding of the directional switching.

* Formerly: Proteins Involved in Axonal Transport and Structure of Neuronal Cytoplasm

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02873-01 LN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunocytochemistry of Neuronal Cytoplasm

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Thomas S. Reese, M.D.

Chief

LN, NINDS

Others: Paul E. Gallant, Ph.D.

Biologist

LN, NINDS

Sven Beushausen, Ph.D.

Visiting Associate

LN, NINDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892. Marine Biobios Laboratory, Woods Hole, MA 02543.

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project determines the structure of neuronal and glial cytoplasm, particularly as it pertains to axoplasmic transport. A protein translocator, kinesin, is responsible for the anterograde organelle movements along microtubules, which are the basis of anterograde fast axonal transport. A high molecular weight protein in squid axoplasm, which we have characterized as a cytoplasmic dynein, transports exclusively in the retrograde direction. The functions of this transport system *in vivo* is also under investigation. Each organelle contacts several microtubules in the axon, so it is the continuous microtubule bundles which constitute the transport pathways down the axon. Much of the pool of kinesin and dynein *in vitro* is in a soluble form and new immunocytochemical methods had to be developed to determine their distributions in the cytoplasm in relation to the transport pathways. This has now been successful and used to show that kinesin is concentrated on the surfaces of organelles and that there is a cytoplasmic pool of free kinesin, suggesting that there may be two different pools of kinesin with different functions, such as promoting interaction of microtubules with the endoplasmic reticulum or with other microtubules. These interactions are likely to be of fundamental importance in the growth, regeneration, and maintenance of axons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02835-02 LN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Subcellular Organization in Excitable Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Evelyn Ralston, Ph.D.	Special Expert	LN, NINDS
Others:	Stefanie Kaech, Ph.D.	Special Volunteer	LN, NINDS
	Jae Bum Kim, B.A.	Biologist	LN, NINDS
	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Bernhard E. Flucher, Ph.D.	Visiting Associate	LN, NINDS

COOPERATING UNITS (if any)

Herman Gordon, Ph.D., Dept. of Anatomy, Univ of Arizona, Tucson, AZ. Thorkil Ploug, M.D., EDMN, DB, NIDDK, Bethesda, MD.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to understand how mRNAs, proteins, and subcellular organelles are distributed and organized into functional domains in excitable cells. We continue to use the model system of hybrid myotubes, based on the mouse muscle cell line C2, described previously. In addition, new tools have been developed in the past year. First, a tissue culture system of low-density rat hippocampal neurons has been established in our laboratory. It will allow us to extend our work to nerve cells. The advantage of this specific system is that individual neurons can be observed in the process of differentiating and acquiring polarity. Second, we have obtained expression of the β -galactosidase cDNA in these neurons. Several approaches were attempted: transfection of plasmid DNA with a commercial reagent (Transfectam), direct injection of DNA, and electroporation. So far, transfection has given the best results in terms of cell survival and optimization of expression. In the near future we will start transfections with a series of plasmids that will help us determine the effect of mRNA stability on protein and mRNA distribution in muscle and nerve cells. Expression of these plasmids has already been tested on C2 muscle cells. For this purpose, a new protocol using transient rather than permanent transfections to form hybrid myotubes, containing a single transfected nucleus in a background of unmodified cells, has been established. The next step will be to determine the distribution of the mRNAs of transfected plasmids by in situ hybridization. Thus, we have several new tools available to study protein and mRNA distribution in muscle and nerve cells. Studies on the distribution of subcellular organelles, especially the Golgi complex, have also progressed. A developmental study of the Golgi complex distribution in muscle cells in vitro and muscle fibers in vivo was completed by an electron microscopy (EM) study carried out in the NINDS EM Facility. It confirmed our light microscopy results that suggested that the Golgi complex was distributed throughout the length of a muscle fiber, both near and away from the neuromuscular junction. This was an important point to establish. Finally, a new collaborative study of the dynamics and changes of distribution of the Golgi complex and associated membranes in muscle in vivo has been started, using the muscle-specific glucose transporter GLUT4 as a marker and combining both light and electron microscopy.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02872-01 LN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Adhesion in Vertebrate Neural Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Judith A. Drazba, Ph.D.

IRTA Fellow

LN, NINDS

Others: Carolyn L. Smith, Ph.D.

Senior Staff Fellow

LNC, NINDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Brain Structural Plasticity

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.3

PROFESSIONAL:

1.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is aimed at characterizing differences in the adhesive patterns and associated growth characteristics of neuronal growth cones on defined substrates. Neurite formation by embryonic retinal ganglion cell neurons was observed using a technique (time-lapse laser scanning interference reflection microscopy) to show local distances of the cell membrane from substrates composed of purified, biologically relevant cell and substrate adhesion molecules. Members of the three major classes of adhesion molecules were tested - the calcium-dependent, calcium-independent, and the immunoglobulin superfamily of molecules. The overall degree to which growth cones bind to different molecular substrates varies widely, but a trend can be seen between reduced adhesivity and increased growth cone motility, suggesting that increasing levels of adhesivity may not be the basis of growth promotion as previously suggested. Analysis of the dynamic changes in adhesion patterns also shows wide variation among substrates. Since growth cones *in vivo* encounter many molecules at the same time, it seems reasonable to suggest that they must be able to integrate these many signals into a response that is suitable for directing them to their appropriate target. We suggest that there is a critical level and, perhaps, pattern of adhesion of a growth cone to its substrate that are necessary for neurites to form and move forward. Experiments will continue to analyze adhesive interactions to molecules in combination and in patterned arrays. We will also begin to investigate the role of cytoskeletal interactions with adhesion molecules on the cell surface to understand how signals in the growth cones' environment may be transduced into behavioral responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02871-01 LN									
PERIOD COVERED October 1, 1991 through September 30, 1992											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Postsynaptic Densities: Mechanisms for Structural Modification											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Ayse Dosemeci, Ph.D.</td> <td style="width: 33%;">Visiting Associate</td> <td style="width: 33%;">LN, NINDS</td> </tr> <tr> <td>Others: Thomas S. Reese, M. D.</td> <td>Chief</td> <td>LN, NINDS</td> </tr> <tr> <td>Paul E. Gallant, Ph.D.</td> <td>Biologist</td> <td>LN, NINDS</td> </tr> </table>			PI: Ayse Dosemeci, Ph.D.	Visiting Associate	LN, NINDS	Others: Thomas S. Reese, M. D.	Chief	LN, NINDS	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
PI: Ayse Dosemeci, Ph.D.	Visiting Associate	LN, NINDS									
Others: Thomas S. Reese, M. D.	Chief	LN, NINDS									
Paul E. Gallant, Ph.D.	Biologist	LN, NINDS									
COOPERATING UNITS (if any)											
LAB/BRANCH Laboratory of Neurobiology, BNP, DIR, NINDS											
SECTION Section on Structural Cell Biology											
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892											
TOTAL STAFF YEARS: 1.8	PROFESSIONAL: 1.8	OTHER: 0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews		
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither									
<input type="checkbox"/> (a1) Minors											
<input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Structural changes in postsynaptic densities may underlie long-term modifications of synaptic activity. The aim of this project is to study the molecular organization of the postsynaptic densities and to explore the potential mechanisms for their modification in response to calcium and other intracellular messengers. Results obtained during the past year using postsynaptic density preparations from cerebral cortex indicate that the <u>calcium calmodulin-dependent protein kinase</u>, which is the most abundant protein in the densities, is a strong candidate for mediating such structural modifications. We show that the densities contain an endogenous <u>phosphatase</u> of type 1 that actively dephosphorylates the calcium calmodulin-dependent protein kinase. When this phosphatase is effectively inhibited, almost all of the postsynaptic density-associated kinase pool becomes phosphorylated. Modification of the postsynaptic density structure in response to elevated levels of calcium may also occur through selective degradation of component proteins by <u>calpain</u>, a calcium-activated protease. Preliminary studies that show different rates of degradation of different component proteins upon treatment of the postsynaptic density preparations with exogenous calpain, support this hypothesis. In the coming year, we shall continue studies on the biochemical and morphological characterization of postsynaptic densities. To study the consequences of the autophosphorylation of the kinase, we shall take advantage of our findings on the use of the phosphatase inhibitor, <u>Microcystin-LR</u>, to obtain a postsynaptic density preparation with a fully phosphorylated pool of calcium calmodulin-dependent protein kinase. The consequences of autophosphorylation and of limited degradation of components by calpain on morphology, protein-protein interactions, and other biochemical properties of the postsynaptic densities will be investigated.</p>											
17-LN/DIR											

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02841-02 LN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of the Endoplasmic Reticulum

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Mark Terasaki, Ph.D.	Senior Staff Fellow	LN, NINDS
Others:	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	Jorge E. Moreira, Ph.D.	Visiting Scientist	LN, NINDS
	Thomas S. Reese, M.D.	Chief	LN, NINDS

COOPERATING UNITS (if any)

L.A. Jaffe, University of Connecticut Health Center, Farmington, CT; C. Sardet, CNRS, Villefranche, France.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The structure and function of the endoplasmic reticulum (ER) in neurons and glia are being investigated using newly developed techniques which make it possible to investigate the dynamic properties of the ER in living cells by video and laser scanning confocal microscopy. New techniques have been developed using the sea urchin egg as a model system. The sea urchin egg has the advantages of being readily available, having a prominent ER, and being easy to microinject. A novel technique for specifically staining ER in living cells shows that the ER undergoes movements in the absence of microtubules, that a striking change in its organization occurs at the time of calcium release, and that the appearance of microtubules has a profound effect on its organization. By use of this technique on fixed cells, the continuity of ER membranes can be determined. We are currently adapting these techniques so they can be applied to understand the organization and dynamics of the ER in neuronal cell bodies, axons and also in muscle cells. A second initiative uses calcium-sensitive fluorescent indicators to investigate calcium regulation by the ER. In a cell-free preparation derived from sea urchin eggs, it was demonstrated that the ER is the source of InsP3-induced calcium release. Cytosolic calcium is being investigated with laser scanning confocal microscope using a novel improvement in fluorescent calcium indicators. Application of these sensitive calcium indicators to the question of the distribution of calcium entering axons is now being investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02842-02 LN
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology of Neural Function in Invertebrate Models*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Sven A. Beushausen, Ph.D.	Visiting Associate LN, NINDS
Others:	Jorge E. Moreira, Ph.D.	Visiting Scientist LN, NINDS
	Thomas S. Reese, M.D.	Chief LN, NINDS
	Howard Jaffe, Ph.D.	Special Expert LNC, NINDS
	Joanne Gutierrez, B.S.	Chemist LCB, NIMH
COOPERATING UNITS (if any) H. Bayley, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545. M. Miller, K. Weiss, J. Brosius, Fishberg Res. Center, Mount Sinai Sch Med, New York, N.Y. I. Kupferman, New York State Psychiatric Institute, N.Y.		
LAB/BRANCH Laboratory of Neurobiology, BNP, DIR, NINDS		
SECTION Section on Structural Cell Biology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892.		
TOTAL STAFF YEARS:	2.0	PROFESSIONAL: 1.5 OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p> The following summary describes four projects that attempt to investigate, at the molecular level, the roles a number of regulatory molecules, including, <u>A kinase</u>, <u>kinesin light chains</u>, the modulatory neuropeptides <u>buccalin</u> and <u>myomodulin</u> and <u>rab 3</u> proteins, play in neural function. Two different invertebrate systems are currently being studied. <u>Synaptic plasticity</u> is being studied as a consequence of changes in the distribution and substrate specificity of A kinase <u>catalytic subunits</u> in response to the application of extracellular stimuli to sensory neural clusters in the sea hare <u>Aplysia californica</u>. Secondly, we have characterized the <u>peptide families</u> of <u>buccalin</u> and <u>myomodulin</u> in an attempt to gain further insights as to how peptide families collectively contribute to pre- or postsynaptic neuromodulation. The molecular characterization of peptide receptors through which <u>signal transduction cascades</u> are activated to affect modulation, for example <u>G protein coupled receptors</u>, is also being actively pursued. The characterization of <u>kinesin light chains</u> in the squid <u>Loligo pealei</u>, is being undertaken to define the <u>diversity of light chains</u> in the nervous system and ultimately to determine <u>function and specificity</u> with regard to intracellular transport, <u>particularly a</u> <u>rnal transport in neurons</u>, of <u>membrane-bound organelles</u>. Similarly, cloning of the small GTP-binding proteins is being pursued to determine the role <u>rab 3a</u>, in particular, plays in synaptic transmission. Although each project is being studied independently, there are aspects of each that directly impinge on another. For example, the effects of <u>myomodulin</u> are believed to be affected through the activation of the <u>A kinase cascades</u>. It is also believed that the activity of kinesin, both heavy and light chains, are <u>regulated</u> via <u>phosphorylation</u> in which A kinase is a likely candidate. The <u>synaptic vesicles</u> that <u>buccalin</u>, <u>myomodulin</u> and <u>rab 3a</u> localize to are carried along the axon via fast axonal transport, a mechanism thought to be facilitated by kinesin. </p>		
*Formerly entitled "Catalytic Subunit Characterization of the cyclic AMP-Dependent Protein Kinases from the Marine Mollusc <u>Aplysia californica</u> ".		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02610-09 LN
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Elemental and Structural Organization of Neurons and Glia*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	S. Brian Andrews, Ph.D.	Section Chief LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief LN, NINDS
	Roger Buchanan, Ph.D.	IRTA Fellow LN, NINDS
	Asher Shainberg, Ph.D.	Visiting Scientist LN, NINDS
	Maureen F. O'Connell, B.S.	Biologist LN, NINDS
COOPERATING UNITS (if any) R.D. Leapman, BEIP, NCCR, NIH, Bethesda, MD. D.M.D. Landis, Case-Western Reserve University, Cleveland, OH. B.D. Trapp, Johns Hopkins University School of Medicine, Baltimore, MD. J.A. Hunt, Lehigh University, Bethlehem, PA.		
LAB/BRANCH Laboratory of Neurobiology, BNP, DIR, NINDS		
SECTION Section on Analytical Cell Biology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892; Marine Biological Laboratory, Woods Hole, MA 02543		
TOTAL STAFF YEARS:	3.0	PROFESSIONAL: 1.8 OTHER: 1.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This two-part project studies the organization and function of specialized membranes in neurons and glia. The first part aims to characterize <u>calcium regulation</u> during synaptic activity in parallel fiber/Purkinje cell synapses of the <u>cerebellar cortex</u>. New frozen sectioning techniques, in combination with <u>scanning transmission electron microscopy</u> (STEM), have permitted studies of coordinated changes in cytoplasmic total calcium which accompany regulation of free <u>intracellular calcium</u> by <u>endoplasmic reticulum</u> (ER). The results show that the (previously described) characteristic calcium concentration states of the ER in <u>dendritic spines</u> are associated with specific cytoplasmic calcium levels. This leads to three distinct states of calcium mobilization, which likely reflect the sequential processes of release from intracellular stores, followed by calcium uptake through membrane channels. In spine-bearing dendrites, there is a class of calcium-rich ER which is not present in spines, suggesting more complex patterns of calcium regulation in <u>dendrites</u>. A new method, based on darkfield <u>mass mapping</u> in the STEM, has been developed for determining the in situ molecular mass of organelles within neuronal processes. This capability has proven valuable for uncovering changes in protein binding and water content that reflect synaptic activity. Structural analysis of directly frozen preparations of various <u>organotypic cultures of hippocampus</u> continues to define the organization of dendrites and spines in the pyramidal cells of this tissue. In Part Two, formation of specialized membranes is studied in the context of myelin assembly. <u>Confocal light microscopy</u> has shown that <u>Schwann cells</u> depend on <u>microtubule-based intracellular transport</u> and assembly of <u>myelin-specific proteins</u>. Moreover, the various myelin proteins synthesized in the perinuclear region are sorted in the trans-Golgi network into distinct <u>transport vesicles</u>, and this process also appears to be directed by microtubules. There are strong and important associations between microtubules and other cytoskeletal components within cytoplasmic channels of the internode; the organization of the Schwann cell ER appears to depend on these interactions. </p>		
*Formerly: "Distribution of Mobile and Structural Components at Chemical Synapses"		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02836-02 LN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural and Elemental Analysis of Macromolecular Assemblies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Brian Andrews, Ph.D.	Section Chief	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	Maureen F. O'Connell, B.S.	Biologist	LN, NINDS

COOPERATING UNITS (if any)

R.D. Leapman, BEIP, NCCR, NIH, Bethesda, MD. J.A. Hunt, Lehigh University, Bethlehem, PA.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Analytical Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892; Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

0.8

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to characterize the shape, molecular weight distribution, and elemental composition of individual macromolecules and macromolecular assemblies. Such assemblies are critical to many cell functions, and their behavior in vitro reflects their function and regulation in intact cells. This project depends on an unique instrument - a low-temperature, high-resolution, field-emission scanning transmission electron microscope (STEM) - for molecular weight mapping and chemical analysis by parallel electron energy loss spectroscopy (PEELS). The development of this instrument, and new preparation techniques for directly-frozen thin films and high-resolution platinum replicas were described last year. Now, the capabilities of this STEM have been extended to ultrathin cryosections of directly frozen tissues. Mass mapping and calcium analysis of sections of cerebellar cortex have characterized two distinct classes of endoplasmic reticulum (ER) in the dendrites of Purkinje cells. In addition, spectrum imaging, a new technique for high-resolution elemental mapping by PEELS, has been implemented and used to image the distribution of calcium-sequestering ER within these dendrites. To measure the distribution of water within Purkinje cell dendrites, a new method based on analyzing the low-loss region of low-dose PEELS map of frozen-hydrated sections has been developed. The combination of low-dose mass mapping and high-dose PEELS spectrum imaging would appear to be a practical method for correlating ion and water fluxes that accompany neuronal activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201-NS-02834-02 LN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Excitation-Contraction Coupling in Muscle

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Bernhard E. Flucher, Ph.D.	Visiting Associate	LN, NINDS
Others:	S. Brian Andrews, Ph.D.	Section Chief	LN, NINDS
	Maureen O'Connell, B.S.	Biologist	LN, NINDS
	Jae Kim, B.S.	Biologist	LN, NINDS
	Asher Shainberg, Ph.D.	Visiting Scientist	LN, NINDS

COOPERATING UNITS (if any)

M.P. Daniels, LGB, NHLBI, NIH, Bethesda, MD. J.A. Powell and J.L. Phillips, Smith College, Northampton, MA. C. Franzini-Armstrong, Penn. Philadelphia, PA.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Analytical Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to determine the molecular mechanisms involved in the assembly of the triad junction between T-tubules and sarcoplasmic reticulum during the development of excitation-contraction (E-C) coupling in skeletal muscle. An immunofluorescence study of the distribution of the α subunit of the skeletal muscle dihydropyridine (DHP) receptor (the putative voltage sensor in E-C coupling) in developing normal muscle and dysgenic (mdg) myotubes in culture suggested that a specific protein-protein interaction of the α subunits of the DHP receptor is involved in the normal organization of the receptor complex in the junctional T-tubules. The coordinated expression of several myofibrillar and membrane markers was compared between normal and dysgenic muscle developing *in vivo* and *in vitro*. Proteins of the I-Z-I complex (α -actin, titin T12) and the sarcoplasmic reticulum (Ca^{2+} -ATPase) organize early while components of the A-band (myosin, titin T30) and the T-tubules assume a cross-striated orientation later in myogenesis. Although dysgenic muscle lags behind in the later phase of sarcomere formation, mutant myotubes can achieve the mature organization in the myofibrils and E-C coupling membranes. *De novo* expression of the DHP receptor α_1 subunit from normal nonmuscle nuclei fused with dysgenic myotubes restored normal functions as well as normal organization of myofibrils and E-C coupling membranes.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-01805-24 LN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Membrane Structure of Astrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Elena Sanovich, Ph.D.

Visiting Associate

LN, NINDS

Others: Milton W. Brightman, Ph.D.

Section Chief

LN, NINDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Brain Structural Plasticity

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.8

PROFESSIONAL:

0.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Astrocytes have glutamate-induced rhythmic fluctuations of Ca^{2+} in their cytosol. The pituicyte, an astrocytic cell in the neural lobe of the pituitary gland, is bathed by neurosecretory peptides: vasopressin, dynorphin and oxytocin released from axon terminals. Is there release of Ca^{2+} into the cytosol of pituicytes in response to stimulation with these peptides? Shifts in the compartmentalization of Ca^{2+} , which fluoresces when it forms a complex with Fluoro-3, is monitored by fluorescence changes detected with video-enhanced microscopy. In pilot experiments, 100 μM or greater amounts of arginine vasopressin, when added to pituicytes that have been preloaded with Fluoro-3 and maintained at room temperature, result in release of Ca^{2+} which pulses once or twice. Nonphysiologic amounts, i.e., 1 mM, of dynorphin and 100 μM of glutamate, which trigger Ca^{2+} release in cerebral astrocytes, do not do so in pituicytes. Do pituicytes respond to osmolarity changes of extracellular fluid? Addition of 0.5 g % sucrose to the cells had no effect on Ca^{2+} release. Repetition of the experiments at 37° C may yield different results. Exposure of pituicytes to 10 μM dynorphin for 5 minutes induces the expression of the "early immediate" proto-oncogene c-fos. The response is diminished by 30 minutes and is over by 3 hours. The other peptides and neurotransmitters were used as well. This project is in abeyance.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02086-19 LN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regeneration Specificity in Transplanted Neural Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	David L. Simpson, M.D.	Special Expert	LN, NINDS
Others:	Elena Sanovich, Ph.D.	Visiting Fellow	LN, NINDS
	Milton W. Brightman, Ph.D.	Section Chief	LN, NINDS

COOPERATING UNITS (if any)

J. Bressler, Kennedy Institute, Baltimore, MD.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Brain Structural Plasticity

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One of two mechanisms may account for the disappearance of naive PC12 pheochromocytoma cells implanted into brain and the retention of PC12 neuronally differentiated by ras-oncogene. As there appears to be no significant difference in major histocompatibility complexes between naive and ras PC12 cells, it is likely that cell surface changes in, e.g., sialic acid, and cell adhesion molecules (CAMs) may modulate cell-cell and cell-substrate adhesion in vivo and thence the persistence of the ras cells in brain. The amount of sialic acid is assessed by the binding of the lectin, wheat germ agglutinin (WGA), on the surface of naive, nerve growth factor (NGF) treated and ras-PC12 cells. The ras cells have significantly more ¹²⁵I-WGA binding than have naive or NGF treated cells. Moreover, WGA blocks regeneration of neurites in NGF exposed PC12 cells but not in ras-PC12 cells. Partially characterized clones of PC12 cells that do not bind WGA, fail to respond morphologically to both NGF and ras treatment. A significant rise in immunoreactivity of protein kinase C, that may phosphorylate proteins associated with the surface molecules, has been detected in PC12 cells 24 hr after infection with ras. Further analysis of surface molecules may support the inference that CAMs rather than histocompatibility complexes affect PC12 cell survival in brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02869-01 LN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Influence of Leukocytes on Neural Regrowth

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Milton W. Brightman, Ph.D.	Section Chief	LN, NINDS
Others:	Nicholas DiProspero, B.S.	Summer IRTA	LN, NINDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Brain Structural Plasticity

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this work is to repeatedly introduce exogenous macrophages (MØ), activated to their secretory state, into rats with a spinal cord injury. The exogenous MØ provide a continual supply of vasogenic and growth factors that might promote regeneration of axons across the lesion. Previously, we showed that exogenous MØ can be introduced into the medulla of a brain upon which is placed a muscle autograft. We now ask whether the introduction of such exogenous, activated MØ and, eventually of monocytes, can promote regeneration of damaged central axons. A crush lesion is made epidurally at the T-5 segment of the spinal cord in adult rats. MØ, activated with lipopolysaccharide to their secretory state when they release their vasogenic and growth factors, are injected intraperitoneally at different times over a 3-week period following the injury. The exogenous, activated MØ, in this preliminary work, are attracted to and accumulate at the injured site. In those rats that had received the MØ 24 hr after injury and repeatedly thereafter, cavitation of the cord was less and the cellular density around the lesion was greater than in untreated rats. Monocytes which survive longer in vivo than do activated MØ, and whose functional states are more readily recognizable, are to be used next.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02144-18 LN
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Blood-Brain Barrier		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Shoichiro Ishihara, M.D.	Visiting Fellow LN, NINDS
Others:	Milton W. Brightman, Ph.D.	Section Chief LN, NINDS
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neurobiology, BNP, DIR, NINDS		
SECTION Section on Brain Structural Plasticity		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	1.4	PROFESSIONAL: 1.4 OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>An important <u>hypothesis</u> of vascular specificity is that a <u>vessel's phenotype</u> is determined by the <u>target tissue</u> being vascularized rather than by the <u>source</u> of the vessel. We have found an <u>exception</u> to this hypothesis. When autografts of skeletal muscle are placed in the choroid plexus of the IV ventricle, some of the vessels that grow into the target muscle graft are not of the muscle type but, rather, of the source: the fenestrated vessels of the choroid plexus. This exception is most apparent and frequent, for unknown reasons, in those rats from which both superior cervical ganglia had been removed in order to ascertain the source of the grafts' innervation. In 4 of 5 <u>ganglionectomized</u> rats, some of the vessels that grew among muscle fibers of the graft, were not of the muscle type but rather fenestrated, like those of the choroid plexus. The source, not the milieu of the target muscle tissue, had determined the structural phenotype of the muscle. In order to see whether the mature graft tissue had lost its ability to induce vessel changes, 18-day-old fetal muscle is being grafted. It is likely that the hypothesis is valid when both the target and source tissues are fetal.</p>		

ANNUAL REPORT

October 1, 1991 through September 30, 1992

Laboratory of Neurochemistry
National Institute of Neurological Disorders and Stroke

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ANNUAL REPORT
October 1, 1991 through September 30, 1992
Laboratory of Neurochemistry, Division of Intramural Research
National Institute of Neurological Disorders and Stroke
Harold Gainer, Ph.D., Chief

The Laboratory of Neurochemistry (LNC) is concerned with the development, structure, and functional organization of the nervous system, with a special focus on molecular mechanisms. The Laboratory is composed of three Sections: Cellular and Developmental Neurobiology, Enzyme Chemistry, and Molecular Neuroscience, a Neurogenetics Unit, and a Peptide and Protein Sequencing Core Facility which serves the entire Division of Intramural Research, NINDS. In general, research projects in the LNC are interdisciplinary and use a wide variety of techniques ranging from the neuroanatomical to the cell and molecular biological. Invertebrate (*Drosophila* and squid) and vertebrate (*Xenopus*, electric eel, and transgenic mice) model systems as well as mammalian tissue-cultured cells are employed in studies of neurogenesis; neuronal migration; neuropeptide gene expression and biosynthesis; voltage- and ligand-gated channels and receptors; cytoskeletal proteins and neuronal structure; and the study of specific enzymes (e.g., Na⁺, K⁺-ATPase, protein kinases).

I. Section on Cellular and Developmental Neurobiology

The Cellular and Developmental Neurobiology Section conducts research on the regulation of two classes of neuron-specific genes: 1) genes constitutively expressed in fully differentiated neurons (e.g., neuropeptide and neurofilament genes); and 2) genes transiently expressed during neurogenesis. We have focussed on neuropeptide and neuronal intermediate filament (neurofilament) gene expression because these molecules are differentially and tightly regulated in various specific regions of the nervous system, and are themselves markers for specific neuronal phenotypes. Some of the questions which are of special interest to us are: 1) What are the developmental influences and molecular mechanisms which underlie cell-specific neuropeptide and neurofilament gene expression? 2) Does electrical activity *per se*, neurotransmitter, neurohormone, and/or growth factor receptor activation directly regulate expression of these genes? 3) Is there coordinate regulation of posttranscriptional and/or posttranslational processing? and 4) What are the roles played by second messengers in the above processes?

Neuroendocrine cells are particularly valuable model systems to study neuropeptide gene expression and biosynthesis, and the basic mechanisms which underlie pulsatile neurosecretion. We study selected neuronal populations in the hypothalamus and peripheral nervous system which we believe are especially good models for the analysis of the regulation of peptidergic phenotype and morphology. These are the luteinizing hormone-releasing hormone (LHRH), arginine vasopressin (AVP), and oxytocin (OT)-synthesizing neurons in the hypothalamus, and the peptidergic sensory neurons in dorsal root and trigeminal ganglia, which express abundant calcitonin gene-related peptide (CGRP) and substance P. The presence of a specific peptidergic phenotype in a given cell is the consequence of a highly regulated progression of complex intracellular events. The first of these events is the developmental commitment to the specific peptide phenotype and later the stable expression of the specific gene which encodes the peptide sequence. While this step is critical and necessary, it is not sufficient to guarantee the biosynthesis of a specific peptide in a given cell. Many posttranscriptional and posttranslational processing steps are involved in fashioning the final biologically active neuropeptide from the

expressed gene product. Thus, the Section focuses both on the developmental and homeostatic regulation of neuropeptide gene expression, as well as the cell biological bases of neuropeptide biosynthesis and secretion. Neuroendocrine cells are also highly specialized to secrete large quantities of peptides usually in a pulsatile fashion. Several relevant questions of interest are: 1) Is pulsatile secretion of neuropeptides due to an endogenous pulse generator? Alternatively, is it driven by properties of the circuit/environmental interactions? 2) Are specific receptors and ion channels responsible for generating pulsatility? 3) What are the mechanisms which synchronize the activities of dispersed neuroendocrine cell populations? 4) Is there presynaptic regulation of secretion?

Over the past year, we have continued to develop an "organotypic" tissue culture methodology and strategy to study the above questions. Single cells in slice-explant cultures derived from postnatal differentiated tissues are studied by immunocytochemistry and *in situ* hybridization histochemistry. We have characterized LHRH mRNA levels and OT mRNA levels in cultured neurons grown in defined media in the presence or absence of the sodium channel blocker, TTX. These conditions were shown to alter mRNA levels while not affecting cell survival, and established basal conditions in which regulation of neuropeptide gene expression can be rigorously studied. In principle, we can determine whether specific stimuli act directly on LHRH and/or OT neurons or via interneurons, i.e., extrinsic versus intrinsic properties. As a test case, the effects of estradiol (E_2) treatment on both LHRH and OT mRNA levels were investigated using these organotypic cultures and this strategy. Analysis of single cell LHRH mRNA levels revealed that the effect of E_2 was dependent on the *in situ* anatomical location of the LHRH cell which was cultured. Cells located in the anterior hypothalamic slice showed increased in LHRH mRNA levels after E_2 exposure. This response was prevented by the presence of TTX, suggesting that the effect of E_2 on LHRH cells occurred via an interneuron. LHRH cells at the level of the diagonal band of Broca also showed an increase in LHRH mRNA levels. However, in these cells the response was observed in the presence or absence of TTX, suggesting that a subpopulation of LHRH cells in this region can respond to E_2 directly. The effects of E_2 on OT mRNA levels are also being analyzed. In addition, we are investigating the effects of different second messenger systems on LHRH and OT gene expression. Using analogs of cAMP and phorbol esters, we are beginning to determine the signal transduction pathways used by these neuropeptide genes.

We have initiated a new approach to an integrated analysis of identified hypothalamic neuronal properties. This involves *in vivo* fluorescent labelling of LHRH, OT, and AVP neurons by retrograde transport of a fluorescent probe from neurohaemal circumventricular organs (CVOs). Neonatal animals are injected with Fast Blue and 2-3 days later are used to generate tissues for organotypic slice cultures. Large numbers of CNS cells are labeled using this method and can be seen in the thinned tissue slices on the day that they are generated. After 14-18 days in culture, labeled cells are still apparent and a subpopulation of these cells has been shown to express OT or LHRH. Patch-clamping has been successfully done in unlabeled cultures and cells marked by Lucifer yellow. We are now combining all aspects of this technique, which will allow us to record from primary OT and LHRH cells having efferents to CVOs *in vivo*. Using this protocol we will characterize electrophysiological and pharmacological properties of both cell types as well as other neuroendocrine cells maintained in organotypic slice cultures.

As noted above, sensory ganglion neurons are potential model systems for the study of both neuropeptide and neurofilament gene expression. Tissue-cultured sensory

neurons derived from embryonic day 15 rat dorsal root ganglia have been analyzed for their expression of seven target genes. These included neurofilament-L, -M, -H, peripherin, α -tubulin, CGRP, and substance P. Analysis for mRNA was done by Northern blot and *in situ* hybridization histochemistry, and analyses of proteins and peptides was by Western blot and immunocytochemistry. The results show robust expression of all seven target genes by these sensory neurons in culture, thereby providing a well characterized and second *in vitro* model system for studying regulation of each of these genes.

Our laboratory's successful cloning of the neurofilament gene in the squid nervous system has provided a new opportunity to evaluate whether axons are capable of *de novo* protein synthesis. PCR analyses of mRNA from isolated axoplasm expressed from the squid giant axon showed that neurofilament mRNA was indeed present in this cellular compartment. Antibodies were made to synthetic peptides containing the amino sequences in the N-terminal regions of all three neurofilament protein subunits in squid. These antibodies are currently being used in immunoprecipitation experiments on newly synthesized proteins in the squid giant fiber ganglion (cell bodies) and the giant axon.

In 1989, we proposed that LHRH neurons arise from an extra-CNS origin (the olfactory placode), and during prenatal development migrate from nasal regions into the forebrain where they establish an adult-like distribution. To date, this is the only known neuronal cell type which undergoes such developmental events. The fact that these cells robustly express their peptidergic phenotype prior to and during migration, makes the LHRH system a useful model of neuronal development. In the mouse, LHRH cells move from the olfactory pit (OP) to an adult-like pattern in 5 days. When development of the LHRH system is interrupted, as we have shown in transgenic mice by targeted tumorigenesis to LHRH neurons or in patients with Kallmann's syndrome, reproductive dysfunction results. Although, the embryonic time window in which cells become committed to the LHRH phenotype, the location of the progenitor cells, as well as the onset of LHRH gene expression has been determined, the factors which dictate this cell-specific gene expression and the mechanism(s) whereby LHRH neurons move, and attain their appropriate anatomical location and efferent projections are presently unknown. To address these issues, we are continuing our investigations on the development of the LHRH system using three different strategies: (a) *in vivo* anatomic correlation of molecules thought to be involved in neuronal migration and identification of structures on which neurons may migrate; (b) *in vitro* explant cultures to manipulate movement of primary LHRH neurons and immortalized LHRH cells; and (c) characterization of the intrinsic properties (receptors, signal transduction pathways, etc.) of embryonic LHRH cells *in vivo* and *in vitro*. We are currently testing the hypothesis that N-CAM and fibronectin direct LHRH movement in nasal regions by using embryonic explants which maintain large numbers of LHRH expressing cells. In addition, we have found that LHRH cells do not posttranslationally modify LHRH to the amidated form *in vivo* until reaching the forebrain. Embryonic LHRH cells derived from nasal regions were able to produce "mature" peptide *in vitro* in the absence of brain tissue, suggesting that this is a time-dependent (and not a spatially-dependent) event.

During the past year, the Neurogenetics Unit has focused its efforts on the characterization of three new genes involved in nervous system development. These genes were identified and cloned by enhancer trap screens and transposon tagging in *Drosophila*. Primary structure analysis has revealed that two encode putative transcription factors expressed in CNS neuroblasts while the third encodes a membrane associated protein found in CNS axons that make up the longitudinal

connectives. All three genes are expressed in the CNS during its development. Mutant analysis of these genes has revealed that each is required for survival. A detailed phenotypic analysis of mutant alleles for one of these genes (*castor*) has shown that it is required for the proper differentiation of those cells which express the gene, a subset of CNS axons. Immunolocalization of the *pollux* protein, *castor*'s close genomic neighbor (separated by 99 base pairs), indicates that *pollux* encodes a membrane-associated protein that is concentrated in the axons which make up the longitudinal CNS connectives. Like *castor*, the third gene, *escargot* encodes a putative transcription factor containing a zinc-binding domain. In the developing CNS, *escargot* gene expression is detected in a subset of ventral cord cells. The Neurogenetics Unit has also continued its search for genes that are regulated by the murine *Hox 13* gene, a putative transcription factor expressed in many tissues during development including the CNS.

II. Section on Enzyme Chemistry

The structure, mechanism and functional regulation of the ATP-dependent cation transport proteins. The ability of cells to use metabolic energy to create and maintain gradients of Na^+ , K^+ , Ca^{2+} and protons depends directly on these transport proteins. In the case of the sodium-potassium pump, some general principles of the transport mechanism and considerable structure information are available, but relatively little is known about the relationships between structure and function. Current studies are directed toward gaining information about these relationships.

A collaborative study of the transient kinetic properties of the sodium pump of the *Electrophorus* electric organ has shown that electric current is generated by the outward transport of sodium ions in each catalytic cycle and that this event occurs just subsequent to phosphorylation.

Steady-state kinetic studies of similar preparations have shown that the sodium selectivity of the pump is markedly increased when Mg^{2+} binds to a distinct site and conformation of the enzyme. Recognition of the large conformational dependence of the selectivity of the sodium-binding domain emphasizes that the ionophoric mechanism of cation pumps is totally different from that of channels. Three sodium ions must bind simultaneously to this domain prior to the transport event. A goal of current experiments is to determine whether the conformationally dependent selectivity is a shared property of all three sites.

Another phase of this project involves studying the isoforms of the sodium pump that are expressed in rat brain tissue. Studies of the selective regulation of the expression of α -subunit isoforms in the pituitary are in progress. A combination of immunohistochemistry and Western blotting has been used to demonstrate that all 3 isoforms are expressed in the posterior pituitary. The anterior lobe expresses the $\alpha 1$ form throughout with a minor population of cells expressing the $\alpha 2$ form. The major cell type of the intermediate lobe expresses only the $\alpha 1$ form. Current studies are measuring the levels of expression of mRNA for each isoform and how this expression may change in the pituitary and hypothalamus of rats in response to water and salt-loading.

The structure and function of the neuronal cytoskeleton. Neurofilaments (NF) are major and unique components of the neuronal cytoskeleton. All three major types (L,M and H) are phosphorylated. NF-M and NF-H contain tandemly repeated sites that become highly phosphorylated during maturation of the nervous system. Multiple protein phosphokinases are operative in this process.

Currently our major efforts in this field are directed toward identification of these kinases. Because it is a long-term maturational process, we have focussed on the second-messenger independent kinases. We have identified three classes of these kinases that can phosphorylate neurofilament proteins: (1) an activity that copurifies with NF and can phosphorylate all three types of NF; (2) microtubule-associated kinase activity that can also phosphorylate all three neurofilament types; and (3) a kinase that phosphorylates a consensus sequence, X(S/T)PXX, that is characteristic of the tandemly repeated sites of NF-H and NF-M. This latter kinase has been cloned and is structurally related to kinases involved in regulation of the cell cycle (cdc2-like kinases). However, because it is present and active in adult brain where cell division and expression of cdc-2 kinase is minimal, its role must be different and it has been designated a neuronal cdc2-like kinase (nclk).

III. Section on Molecular Neuroscience

The Section uses molecular genetic techniques to explore the structure, function and regulation of ligand and voltage-gated signal-transduction mechanisms in the nervous system. In the past year, this Section focussed its studies on the mammalian bombesin-like peptides, gastrin-releasing peptide (GRP) and neuromedin B (NMB), which are known to be neuromodulators, secretagogues, and mitogens in different biologic contexts. Due to changes in focus and personnel, the future directions of the Molecular Neuroscience Section center on studies of the diversity and neural functions of voltage-gated calcium channels and ligand (GABA)-gated channels and receptors.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1-NS-00813-31 LNC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Enzymological Aspects of Neural Functions

P.I.: R. Wayne Albers, Ph.D.

Others: William T. Link, Ph.D.

Paul M. Rowe, Ph.D.

Section Chief

Senior Staff Fellow

Senior Staff Fellow

LNC, NINDS

LNC, NINDS

LNC, NINDS

COOPERATING UNITS (if any)

J.P. Froehlich, Ph.D., NIA, NIH, Baltimore, MD;

K. Fendler, Max-Planck-Institut für Biophysik, Frankfurt, FRG

LAB/BRANCH

Neurochemistry, BNP, DIR, NINDS

SECTION

Enzyme Chemistry

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.5

PROFESSIONAL: 2.5

OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is comprised of research into the structure and function of ion transport systems. There are currently three active subprojects: 1) Transient kinetics: A collaborative study with Froehlich and Fendler. The source of the transmembrane current that is generated by phosphorylation of the sodium pump has been identified as either the major conformational transition or the dissociation of Na^+ that immediately succeeds this event; 2) Studies of the sodium selectivity of different stages of the sodium pump mechanism. Steady-state phosphorylation kinetics have been employed to identify a role for Mg^{2+} in establishing the sodium selectivity of the ionophoric domain of the sodium pump and to correlate this with the conformational state of the pump; and 3) Expression and localization of sodium pump isoforms in the nervous system. A study of the regulation and expression of isoforms of the Na,K - ATPase utilizing oligonucleotides and site-directed antibodies (raised against synthetic peptides) as identifying probes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02723-06 LNC																		
PERIOD COVERED October 1, 1991 through September 30, 1992																				
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Peptides in the Adult and Developing Vertebrate Nervous Systems																				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Harold Gainer, Ph.D.</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">LNC, NINDS</td> </tr> <tr> <td>Co-PI: Susan Wray, Ph.D.</td> <td>Senior Staff Fellow</td> <td>LNC, NINDS</td> </tr> <tr> <td>Others: Margi Goldstein, Ph.D.</td> <td>Senior Staff Fellow</td> <td>LNC, NINDS</td> </tr> <tr> <td>Sharon Key, B.S.</td> <td>Biologist</td> <td>LNC, NINDS</td> </tr> <tr> <td>Kiyoshi Kusano, Ph.D.</td> <td>Visiting Scientist</td> <td>LNC, NINDS</td> </tr> <tr> <td>Christopher Flores, Ph.D.</td> <td>PRAT Fellow</td> <td>LNC, NINDS</td> </tr> </table>			PI: Harold Gainer, Ph.D.	Chief	LNC, NINDS	Co-PI: Susan Wray, Ph.D.	Senior Staff Fellow	LNC, NINDS	Others: Margi Goldstein, Ph.D.	Senior Staff Fellow	LNC, NINDS	Sharon Key, B.S.	Biologist	LNC, NINDS	Kiyoshi Kusano, Ph.D.	Visiting Scientist	LNC, NINDS	Christopher Flores, Ph.D.	PRAT Fellow	LNC, NINDS
PI: Harold Gainer, Ph.D.	Chief	LNC, NINDS																		
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Others: Margi Goldstein, Ph.D.	Senior Staff Fellow	LNC, NINDS																		
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Kiyoshi Kusano, Ph.D.	Visiting Scientist	LNC, NINDS																		
Christopher Flores, Ph.D.	PRAT Fellow	LNC, NINDS																		
COOPERATING UNITS (if any) Dr. WJ Schwartz, University of Massachusetts, School of Medicine; Dr. WS Young, NIMH, NIH; Dr. M Castel, Hebrew University, Israel																				
LAB/BRANCH Laboratory of Neurochemistry																				
SECTION Cellular and Developmental Neurobiology																				
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892																				
TOTAL STAFF YEARS: 1.80	PROFESSIONAL: 1.30	OTHER: 0.5																		
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews											
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither																		
<input type="checkbox"/> (a1) Minors																				
<input type="checkbox"/> (a2) Interviews																				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Neuroendocrine cells</u> express high levels of <u>neuropeptide genes</u> and thus are useful models to examine regulation of <u>gene expression</u> in the CNS. We have found that a wide variety of postnatal neuroendocrine cells survive and express specific neuropeptide genes in <u>organotypic slice-explant cultures</u>. We have characterized <u>luteinizing hormone-releasing hormone neurons (LHRH)</u> and <u>oxytocin (OT) neurons</u> in these cultures maintained in serum-free media, in the presence or absence of <u>tetrodotoxin (TTX)</u>. Using defined media and TTX, we examined the effects of <u>estradiol (E₂)</u> treatment on peptide mRNA levels in LHRH neurons and OT neurons using <u>semiquantitative in situ hybridization histochemistry</u>. Results indicate that E₂ acts differentially on LHRH gene expression, depending on the anatomical location of the LHRH cell examined. For most LHRH cells, the effect of E₂ was blocked by TTX, indicating that the action of E₂ on LHRH gene expression was <u>via an interneuron</u>. However, in one anatomical region, the effect of E₂ was maintained in the presence of TTX, suggesting that a small subpopulation of LHRH cells can respond to E₂ directly. We are currently investigating this phenomenon <u>in vivo</u> and <u>in vitro</u> by analysis of neuronal phenotypes expressing <u>E₂-receptor mRNA</u>. In addition, using these cultures in defined media \pm TTX, we are now examining the <u>signal transduction pathways</u> active in these peptidergic neurons. Currently, we are studying the effects of <u>forskolin</u> and/or <u>phorbol 12-myristate, 13-acetate</u> treatment on both LHRH and OT mRNA maintained in <u>organotypic cultures</u>. Analysis of these explants are currently being done. Finally, by <u>retrograde labeling</u> cells which project to circumventricular organs <u>in vivo</u> and then generating cultures, we are able to identify a subpopulation of neuroendocrine cells <u>in situ</u>. Using this technique, we are patch-clamping, recording and marking neuroendocrine cells whose efferent projections are known. After characterizing the <u>electrophysiological properties</u> of these cells we will identify their neuropeptide phenotype. </p>																				
8 - LNC/DIR																				

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-N5-02724-06 LNC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms in Neuronal Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Harold Gainer, Ph.D.	Chief	LNC, NINDS
Others:	Margi Goldstein, Ph.D.	Senior Staff Fellow	LNC, NINDS
	Shirley House, B.S.	Biologist	LNC, NINDS
	Harish C. Pant, Ph.D.	Research Chemist	LNC, NINDS
	Christopher Flores, Ph.D.	PRAT Fellow	LNC, NINDS
	Philip Grant, Ph.D.	Special Expert	LNC, NINDS

COOPERATING UNITS (if any)

Antonio Guiditta, Ph.D., Institute of Biophysics, Naples, Italy; Michael Tytell, Ph.D., Wake-Forest University, Durham, NC

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Cellular and Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFFYEARS:

2.16

PROFESSIONAL:

1.66

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Tissue cultured sensory neurons derived from embryonic day 15 rat dorsal root ganglia were analysed for their expression of seven target genes. These included neurofilament-L, -M, -H, peripherin, α -tubulin, CGRP and substance P. Analysis for mRNA was done by Northern blot and in situ hybridization histochemistry, and analyses of proteins and peptides by Western blot and immunocytochemistry. The results show robust expression of all seven target genes by these sensory neurons in culture, thereby providing a new model system for study of the regulation of each of these genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1-NS-02725-06 LNC			
PERIOD COVERED October 1, 1991 through September 30, 1992					
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Calcium Metabolism and Protein Phosphorylation in Neuronal Systems					
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> P.I.: Harish C. Pant, Ph.D Others: Philip Grant, Ph.D William T. Link, Ph.D Howard Jaffe, Ph.D. Kurudunje T. Shetty, Ph.D. Alexander Wheaton </td> <td style="width: 33%; vertical-align: top;"> Research Chemist Special Expert Senior Staff Fellow Special Expert Visiting Scientist Biologist Lab Technician </td> <td style="width: 33%; vertical-align: top;"> LNC, NINDS LNC, NINDS LNC, NINDS LNC, NINDS LNC, NINDS LNC, NINDS </td> </tr> </table>			P.I.: Harish C. Pant, Ph.D Others: Philip Grant, Ph.D William T. Link, Ph.D Howard Jaffe, Ph.D. Kurudunje T. Shetty, Ph.D. Alexander Wheaton	Research Chemist Special Expert Senior Staff Fellow Special Expert Visiting Scientist Biologist Lab Technician	LNC, NINDS LNC, NINDS LNC, NINDS LNC, NINDS LNC, NINDS LNC, NINDS
P.I.: Harish C. Pant, Ph.D Others: Philip Grant, Ph.D William T. Link, Ph.D Howard Jaffe, Ph.D. Kurudunje T. Shetty, Ph.D. Alexander Wheaton	Research Chemist Special Expert Senior Staff Fellow Special Expert Visiting Scientist Biologist Lab Technician	LNC, NINDS LNC, NINDS LNC, NINDS LNC, NINDS LNC, NINDS LNC, NINDS			
COOPERATING UNITS (if any) Dr. James F. Battey, LBC, NCI; Dr. Mark R. Hellmich, LBC, NCI; James M. Way, Biologist, LBC, NCI					
LAB/BRANCH Neurochemistry, BNP, DIR, NINDS					
SECTION Enzyme Chemistry					
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892					
TOTAL STAFF YEARS: 3.8	PROFESSIONAL: 3.1	OTHER: 0.7			
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews					
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Neurofilament</u> proteins (NFPs) are found exclusively in the nervous system and contain (especially in middle, NF-M and high, NF-H subunits) large numbers of <u>serine phosphorylation</u> sites. Multiple <u>kinase</u> systems are operative in phosphorylating these sites. Our initial aim is to identify these specific kinases in the nervous system. In this regard, we have characterized the following three different classes of second messenger-independent kinases from rat spinal cord which phosphorylate NFPs: 1) casein kinases: <u>casein kinase I</u> (CKI)-like activity appears to be associated with NF preparations isolated from rat spinal cord. CKI, purified from rat spinal cord, phosphorylates all three NF-subunits with a preference for NF-H. <u>Casein kinase II</u> (CKII), on the other hand, poorly phosphorylates NF-H but has higher affinity for NF-M and NF-L; 2) <u>Microtubule-associated protein kinase</u> (MAP kinase)-like activity phosphorylates all three subunits. However, these kinases do not phosphorylate dephosphorylated NFs, nor a tandemly repeated sequence motif containing lys-ser-pro (KSP) present in NF-H and NF-M where these serine residues are extensively phosphorylated; 3) <u>P³⁴cdc2-like kinase</u> (KSP) kinase: this kinase appears to phosphorylate some of the multiple repeats of KSP sequences present in NF-M and NF-H. The consensus sequence recognized by this kinase is XS/TPXK. The properties of this kinase show that it is closely related to <u>P³⁴cdc2 kinase</u> which is known to be involved in the regulation of the cell cycle. This observation implies a different role for neuronal cdc2-like kinases(s) in the adult nervous system where cell division is minimal. We have also taken a molecular biological approach to study the cdc2-like kinase in the nervous system and have cloned and structurally characterized a P³⁴cdc2-like kinase. A low stringency screening procedure was used to screen a rat brain cDNA library using a mouse P³⁴cdc2 cDNA as a probe. A cDNA was identified and named <u>neuronal cdc2-like kinase</u> (nclk) based on its sequence similarity to P³⁴cdc2 and its predominantly neuronal expression. </p>					

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02753-04 LNC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of the Prepro GRP Gene

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James F. Battey, M.D., Ph.D.

Section Chief

LNC, NINDS

Others: Zahra Fathi, Ph.D.

IRTA Fellow

LNC, NINDS

Etsuko Wada, M.D., Ph.D.

Visiting Fellow

LNC, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Neurochemistry, DIR, BNP, NINDS

SECTION

Molecular Neuroscience

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was terminated during FY92, because the principal investigator left NINDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-NS-02774-04 LNC
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Analysis of Mammalian Bombesin Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: James Battey, M.D., Ph.D. Others: Zahra Fathi, Ph.D. Hagit Shapira, Ph.D. Etsuko Wada, M.D., Ph.D. James Way, B.S.	Section Chief IRTA Fellow Visiting Fellow Visiting Fellow Biologist	LNC, NINDS LNC, NINDS LNC, NINDS LNC, NINDS LNC, NINDS
COOPERATING UNITS (if any) Richard Feldman, Ph.D., Triton Biosciences (CRADA-90-004-NS), 1501 Harbor Way Parkway, Alameda, CA 94501; Robert T. Jensen, M.D., Digestive Diseases Branch, NIDDK, NIH; Edward Sausville, M.D., Ph.D., LBC, DTP, DCT, NCI		
LAB/BRANCH Neurochemistry, DIR, BNP, NINDS		
SECTION Section on Molecular Neuroscience		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.25	OTHER: 0.25
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Previously, we cloned and characterized two pharmacologically distinct <u>bombesin receptors</u> (GRP-R and NMB-R) with high affinity for <u>gastrin-releasing peptide (GRP)</u> or <u>neuromedin B (NMB)</u>, respectively. Recently, we isolated and characterized a third distinct mammalian receptor for bombesin peptides (BRS-3) from cultured human lung cancer cells. This receptor is specifically activated by bombesin peptides after expression in <u>Xenopus oocytes</u>. However, higher concentrations of peptide ($> 1 \mu\text{M}$) are needed to reproducibly elicit the response, indicating that a high-affinity bombesin ligand has not as yet been determined for BRS-3. Receptor activation results in elevation of intracellular calcium, presumably coupling through a G_q-like heterotrimeric G-protein which activates phospholipase C-beta 1. BRS-3 is selectively expressed in <u>human lung cancer cells</u> and secondary spermatocytes, but neither in the GI tract nor CNS, and maps to human chromosome X. Receptor chimeras constructed between the GRP-R and NMB-R map the domain responsible for high-affinity NMB-binding and effector signalling to transmembrane segment 5.</p> <p>This project has been transferred to Project No. Z01-CM-07314-01 LBC, NCI and is entitled "Molecular Cloning of Bombesin Receptors."</p>		
12 - LNC/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1-NS-02757-05 LNC
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Analyses of Peptide Receptors and Peptidergic Neuronal Properties		
P.I.: Kiyoshi Kusano, Ph.D. Others: Harold Gainer, Ph.D. Susan Wray, Ph.D. Shirley House, B.S.	Visiting Scientist Laboratory Chief Senior Staff Fellow Biologist	LNC, NINDS LNC, NINDS LNC, NINDS LNC, NINDS
COOPERATING UNITS (if any)		
LAB/BRANCH Neurochemistry, BNP, DIR, NINDS		
SECTION Cellular and Developmental Neurobiology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS: 1.35	PROFESSIONAL: 0.85	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> SUMMARY WORK: <u>Luteinizing hormone-releasing hormone (LHRH), oxytocin (OT) and vasopressin (VP)</u> secreting cells in the <u>rodent hypothalamus</u> were selected for studying of the biophysical mechanisms involved in hormone secretion. <u>Olfactory placodes</u> from mouse embryos (day 16) were isolated and cultured on glass cover-slips for 2-3 weeks in defined medium. <u>Hypothalamic slices</u> of postnatal day 6 rat pups, which had been prelabelled with the vital fluorescent dye Fast Blue by intraperitoneal injection and retrograde transport were plated on glass cover-slips and cultured for 2-3 weeks. Both <u>patch electrode voltage- and current-clamp techniques</u> were applied. Electrical activities of putative LHRH-cells which emigrated from the olfactory placode displayed spontaneous spike discharges which were accompanied by transsynaptic activities. The soma region of these cells possessed initial transient <u>sodium current</u> (I_{Na}), <u>potassium currents</u> ($I_{K(V)}$, $I_{K(A)}$) and <u>calcium current</u> ($I_{Ca(t)}$). Following electrophysiological analyses, the recorded cells were injected with <u>Lucifer Yellow (LY)</u> for frequent identification by immunocytochemical staining for LHRH cells. Electrical activities of Fast Blue-stained cells in the <u>supraoptic nucleus (SON)</u> and <u>paraventricular nucleus (PVN)</u> also exhibited spontaneous regular and irregular discharges. Some cells displayed intermittent burst discharges. Some of these discharges were endogenous, but transsynaptically driven discharges were also recorded. Following electrophysiological analyses, these LY-marked cells were also processed for their phenotypic identification (i.e., LHRH-, OT-, or VP-cells) by <u>immunocytochemistry</u>. An <u>intracellular Ca^{2+}-imaging</u> and a high-time resolution <u>single cell Ca^{2+}-photometry</u> system was constructed for the simultaneous measurement of electrophysiological activities and intracellular Ca^{2+} concentration changes in a single cell or a population of cells. </p>		
13 - LNC/DIR		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02824-02 LNC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ontogeny of the Luteinizing Hormone Releasing Hormone (LHRH) System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Susan Wray, Ph.D.	Senior Staff Fellow	LNC, NINDS
Other:	Sharon Key, B.S.	Biologist	LNC, NINDS
	Kiyoshi Kusano, Ph.D.	Visiting Scientist	LNC, NINDS
	Susan Fueshko, Ph.D.	IRTA Fellow	LNC, NINDS

COOPERATING UNITS (if any)

S Radovick, M.D., Case Western Reserve School of Medicine, Cleveland, Ohio; K Mahon, Ph.D., NICHD, NIH; WP Hayes, NICHD, NIH; J Battey, LBC, NCI, NIH

LAB/BRANCH

Laboratory of Neurochemistry, DIR, NINDS

SECTION

Section on Cellular and Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.45

PROFESSIONAL:

0.95

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

LHRH neurons are derived from the olfactory placode and migrate into the brain. The factors which dictate cell-specific LHRH gene expression and the migratory mechanism(s) used by the LHRH neurons during their movement in the CNS are currently unknown. Using *in vivo* and *in vitro* models, we are examining the prenatal development of the LHRH system. We are testing the hypothesis that N-CAM and fibronectin direct LHRH movement in nasal regions by using embryonic explants which maintain large numbers of LHRH expressing cells. In addition, we have found that LHRH cells do not posttranslationally modify LHRH to the amidated form *in vivo* until reaching the forebrain. Embryonic LHRH cells derived from nasal regions were able to produce the "mature" peptide *in vitro* in the absence of brain tissue, suggesting that this is a time-dependent (but not spatially-dependent) event.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02698 07 LNC												
PERIOD COVERED October 1, 1991 through September 30, 1992														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biology of Mammalian Homeodomain Proteins*														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Ward F. Odenwald, Ph.D.</td> <td style="width: 35%;">Senior Staff Fellow</td> <td style="width: 15%;">LNC, NINDS</td> </tr> <tr> <td>Others:</td> <td>Peter Vos, Ph.D.</td> <td>IRTA Fellow</td> <td>LNC, NINDS</td> </tr> <tr> <td></td> <td>Shang-Ding Zhang, M.D.</td> <td>Visiting Fellow</td> <td>LNC, NINDS</td> </tr> </table>			PI:	Ward F. Odenwald, Ph.D.	Senior Staff Fellow	LNC, NINDS	Others:	Peter Vos, Ph.D.	IRTA Fellow	LNC, NINDS		Shang-Ding Zhang, M.D.	Visiting Fellow	LNC, NINDS
PI:	Ward F. Odenwald, Ph.D.	Senior Staff Fellow	LNC, NINDS											
Others:	Peter Vos, Ph.D.	IRTA Fellow	LNC, NINDS											
	Shang-Ding Zhang, M.D.	Visiting Fellow	LNC, NINDS											
COOPERATING UNITS (if any)														
LAB/BRANCH Laboratory of Neurochemistry														
SECTION Cellular and Developmental Neurobiology (Neurogenetics Unit)														
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892														
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"><input type="checkbox"/> (a) Human subjects</td> <td style="width: 33%;"><input type="checkbox"/> (b) Human tissues</td> <td style="width: 33%;"><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews					
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither												
<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) *This project has been transferred to Project # Z01-NS-02820-03 LNC under the title: "Cloning and Functional Analysis of Genes Active in Neurogenesis."														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02820-03 LNC
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cloning and Functional Analysis of Genes Active in Neurogenesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Ward F. Odenwald, Ph.D.	Senior Staff Fellow LNC, NINDS
Others:	Dervla Mellerick-Dressler, Ph.D.	Staff Fellow LNC, NINDS
	Shang-Ding Zhang, M.D.	Visiting Fellow LNC, NINDS
	Peter Vos, Ph.D.	IRTA Fellow LNC, NINDS
COOPERATING UNITS (if any) J. Kassis, Ph.D., CBER, DBB; H. Arnheiter, M.D., LVMP, NINDS; W. Mitchell, D.V.M., Ph.D., LNEP, NINDS		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION Cellular and Developmental Neurobiology (Neurogenetics Unit)		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	4	PROFESSIONAL: 4 OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The mechanisms that underlie the orderly sequential changes in <u>gene expression</u> during <u>neurogenesis</u> are unknown. It is now clear that these communications regulate neuroblast development, axonal growth and guidance, and ultimately synapse formation. The molecular machinery that relays this information is the foundation upon which neuronal diversity and function is based.</p> <p>Over the past two years, we have searched for, identified and cloned three new genes involved in the neurogenesis of the fruit fly, <i>Drosophila melanogaster</i>. This past year, we have focused our efforts on characterization of these genes by studying their expression, generating multiple mutant alleles for each, and analyzing the consequences of their mutations. Recently, we have used a recombinant protein to generate polyclonal antibodies against one of the encoded proteins. We plan to use this information and these newly acquired tools to study nervous system development in <i>Drosophila</i> and ultimately search for functionally related genes in mammals.</p> <p><u>Homeodomain proteins</u> represent a highly conserved family of regulatory factors that play an important role in establishing the body plan of metazoans. To understand the biologic function of homeodomain proteins, it is necessary to identify the genes whose expression is regulated by these transcription factors. To determine the <i>in vivo</i> biofunction of the murine <i>Hox 1.3</i> homeodomain protein, i.e., identify genes that are regulated by this putative transcription factor, we are screening cDNA libraries constructed from transgenic mice that contain an inducible <i>Hox 1.3</i> transgene with subtracted cDNA probes.</p>		

ANNUAL REPORT

October 1, 1991 through September 30, 1992
Laboratory of Neurophysiology
Basic Neurosciences Program, DIR
National Institute of Neurological
Disorders and Stroke

Research Summary II-V

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Annual Report
October 1, 1991 through September 30, 1992

Laboratory of Neurophysiology, BNP, DIR
National Institute of Neurological Disorders and Stroke

Jeffery L. Barker, M.D., Chief

The Laboratory of Neurophysiology's research program has been divided primarily among well-established patch- and micro-electrode recording of cellular physiology and associated signal transduction mechanisms and relatively novel strategies involving optically-based methods that expedite the rate at which observations can be obtained, albeit indirectly. This spectrum of complementary and contemporary techniques in neurobiology extends from instantaneous high-fidelity recording of microscopic events at the single channel level to a telescopic perspective of physiologic signals distributed among entire cellular populations.

The broad and long-term goal of this program is to systematically elucidate details regarding the development, differentiation and cellular distribution of specific circuits within the central nervous system (CNS) and, eventually, between the CNS and peripheral pituitary and immune tissues, which are targets of centrally-derived signals and may even be signal generators transmitting to CNS circuits. All CNS cells and their peripheral targets exhibit receptors, ion channels, and an emerging, complex array of signal transduction mechanisms. Multidisciplinary study of the differentiation of specific transmitters, receptors, ion channels and signal transduction mechanisms during CNS development should reveal fundamental insights into the complex process of distributing such mechanisms among cells and their roles both in differentiating and in differentiated circuits. Such insight should provide a physiological basis for determining the cellular and molecular basis of experimentally induced pathologies designed to mimic clinical conditions involving the CNS and its target tissues.

All of the current projects involve cellular or molecular levels of study and most are focussed on the developmental appearance of GABAergic cells and circuits in the rat CNS. By focussing on GABAergic signals with the multidisciplinary strategy implemented over the last 10 years, we have discovered that: 1) transcripts encoding specific GABA_A receptor subunit proteins appear during the very earliest period of postmitotic differentiation in a characteristic sequence at different levels of the neuraxis in parallel with the emergence of transcripts encoding two GABA-synthesizing enzymes; 2) GABA is secreted in a continuous manner from the majority of all embryonic neurons; 3) three distinct GABA receptor-coupled changes in excitability can be detected that involve different concentration requirements, time courses and signal transduction mechanisms; and 4) GABA receptors play a facilitatory role in neuroblast migration. Of particular interest is the remarkable observation that Zn^{2+} , a naturally occurring "trace" metal critical to CNS development, rapidly organizes the continuous secretion of GABA into all-or-none transients that are facsimiles of synaptic signals. Equally insightful has been the resolution of a dynamic process underlying fast GABAergic transmission at synapses. The amplitude and kinetics of the signal recorded postsynaptically can now be explained in terms of the rate of release of identical amounts of GABA from

presynaptic terminals. Thus, we have begun to formulate a new concept of fast synaptic transmission that extends the original "quantal" theory and may in fact explain the modulation of many fast-transmitting synapses independent of the transmitter molecule or its signal, since a dynamic formulation accounts for signals recorded at other synapses. Conceptually, these diverse strategies and lines harmonize to allow quantitative resolution of receptor functions, ion channel expression and signal transduction mechanisms. The strength of the Laboratory's research clearly lies in the broad spectrum of contemporary and innovative strategies established at single-channel, single-cell, circuit, network and population levels, and the opportunity for multidisciplinary and collaborative study into the physiology of intercellular communication emerging in the embryonic CNS.

Several accomplishments this year have long-lasting implications with regard to the role of γ -aminobutyric acid (GABA) and its receptors during the differentiation of embryonic neurons into circuits, which, over the past three years, has become a major focus of LNP research activity.

Drs. Wu Ma, Michael Poulter, Paul Saunders, Anne Schaffner and Ms. Toby Behar, in collaboration with Drs. Lynn Hudson (LMVP, NINDS), Larry Mahan (LCB, NIMH) and Peter Laing (Department of Immunology, Queen's Medical Centre, University Hospital, Nottingham, England) have used *in situ* hybridization techniques in the embryonic rat CNS to describe the advent and anatomical distribution of transcripts encoding both GABA-synthesizing (GAD) enzymes and protein subunits that form Cl^- ion-selective GABA receptors. GAD and GABA receptor transcripts appear in parallel during the very earliest period of postmitotic neuronal differentiation and are expressed by a subpopulation of cells that is still actively proliferating. Although proliferative elements may express these genes, GABA cannot be detected in cells by immunocytochemical methods until neuroblasts have migrated and assumed anatomic sites conducive to differentiation. GABA and a specific GAD isoenzyme colocalize in many central neurons during differentiation of neurites and synapses. During this period, transmitter and enzyme are widely distributed throughout the differentiating cell body and its processes. After synaptogenesis is completed, GABA expression becomes restricted to specific subpopulations where it colocalizes with another GAD isoenzyme in discrete, vesicular organelles. Taken together, the results predict widespread, fundamental roles for GABA during CNS development.

Functional effects of GABA during the embryonic period have been studied by Drs. Marc Walton and Mr. Neil Hardegen using digital video microscopy and image analysis applied to cultured neurons, by Drs. Dragan and Irina Maric and Anne Schaffner and Ms. Susan Smith using flow cytometry applied to suspended cells and by Drs. Jean Vautrin, Alexander Valeyev, Ruggero Serafini, Michael Poulter and Jean-Marc Mienville using patch recordings of cultured cells and acutely prepared sections. GABA, applied exogenously, affects membrane potential and cytoplasmic Ca^{2+} levels in multiple ways beginning in the earliest part of the postmitotic period, and perhaps before. GABA polarizes neurons at different concentrations via different transduction mechanisms that involve either Cl^- or K^+ conductances. Neurons themselves are polarized by ambient GABA, which is actively secreted to mediate autocrine and paracrine functions. By blocking GABA's depolarizing actions Ms. Behar and Dr. Schaffner have eliminated auto- and paracrine roles for GABA on migrating neuroblasts, revealing that GABA and depolarizing actions are critical for migration.

Drs. Vautrin and Valeyev have found that the naturally occurring trace metal Zn, which is necessary for proper CNS development, blocks some Cl⁻ ion selective GABA receptors and alters the process of GABA secretion. Remarkably, within seconds, Zn synchronizes the continuous release of GABA into all-or-none, synaptic-like transients. Dr. Vautrin has recorded thousands of fast GABAergic transients transmitted at synapses differentiating between cultured embryonic central neurons. At the highest level of experimental resolution, Dr. Vautrin has recorded the most elementary signal transmitted, which involves all-or-none activation of 5-10 Cl⁻ ion selective GABA receptors. Using the properties of this unitary signal, Dr. Vautrin can model all of the spontaneously occurring and evoked transients recorded at GABAergic synapses. The model is based on the relative rate of release of unitary packets of GABA. Well-synchronized (high-frequency) release generates large-conductance synaptic signals whose amplitudes and rise-times are normally distributed, according to the classical tenets of quantal theory. Small-conductance events can be mimicked by low-frequency, less-synchronized release, which give rise to quite variable amplitudes and rise-times whose distributions are skewed, not normal. Since other central and peripheral synapses that transit rapidly also exhibit small- and large-conductance, all-or-none transients, it is likely that frequency modulation of unitary release from presynaptic sites can predict the postsynaptic properties of other signals generated by other transmitters acting via receptors coupled to other ion channels. This new model of fast transmission extends the classic "quantal" theory and predicts that changes in fast signal properties, as occurs naturally during both synaptogenesis and aging, and experimentally during intense stimulation of well-differentiated synapses in a "long-term" (hours) manner, are due to frequency modulation of transmitter release from presynaptic sites.

The Drs. Maric have used a discontinuous buoyant density gradient to separate cellular and subcellular elements of the embryonic CNS into subpopulations. Labelling of proliferative cells in a pulse-chase paradigm with a thymidine analog (BrdU) followed by FACS analysis has demonstrated that pre- and postmitotic elements have characteristic buoyant densities. The Marics have used the pulse-chase labelling strategy in collaboration with Dr. Ma to locate these pre- and postmitotic cells in tissue sections. Comparison of labelled sections and suspensions taken from the same litter of embryos places the cells of specific buoyancy in their natural anatomical distribution *in vivo*. Gradient separation is a valuable preparative technique for simplifying the growing complexity of the developing CNS into an experimentally tractable number of subpopulations isolated from anatomical zones that reflect cellular proliferation, migration and differentiation. The Marics along with Dr. Schaffner and Ms. Smith have begun to study the cytoplasmic and membrane properties of gradient-prepared cells. It is already evident from preliminary experiments that stereotypical and reproducible patterns of specific cellular properties can be observed as cells develop more cytoplasm and become more buoyant. Gradient-prepared cells have also been analyzed by Dr. Roland Somogyi for genes encoding several Ca²⁺/lipid dependent-isoenzymes (protein kinase C), which are involved in a myriad array of cellular functions and signal transductions. Dr. Somogyi has used the polymerase chain reaction (PCR) to amplify these PKC genes for surveying separated subpopulations on blots. PKC genes show constant and variable expressions during CNS development. Ms. Smallwood has used gradient separation to analyze the development and distribution of amino acids. Thus, gradient separation can now be used routinely in conjunction with the other resources and strategies established in the LNP to expedite investigations into the complex process of differentiating functional circuits from single nerve and glial cells.

Drs. Ralph Nelson, Michael Freed and Arnaldo Lasansky have continued to make advances in their studies of retinal physiology using in vitro preparations, which are considerably more complex than single cells. All of them have focussed on the role(s) that GABA plays in different retinal networks using electrode recordings from relatively intact retinal preparations. They have found multiple and novel effects of GABA in these well-differentiated preparations. Dr. Tom Smith has continued to quantify the borders of cells developing in culture or differentiated in vivo. His results indicate that certain, complex properties of cellular structure can be quantified quite precisely.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02019-20 LNP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Physiological Properties Developing on CNS Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J.L. Barker, Chief, LNP, BNP, DIR, NINDS. Others (LNP, BNP, DIR, NINDS): A.E. Schaffner, Biologist; M.K. Walton, Senior Staff Fellow; R. Somogyi, Senior Staff Fellow; A. Y. Valeyev, Visiting Scientist; J Vautrin, Visiting Scientist; J-M. Mienville, Visiting Fellow; M.O. Poulter, Visiting Fellow; Q.Y. Liu, Visiting Fellow; H.M. Saunders, IRTA Fellow; R. Serafini, Special Volunteer; N. Hardegen, Chemist; V. Smallwood, Bio Lab. Technician.		
COOPERATING UNITS (if any) G.D. Lange (ICS, NINDS); L. Mahan (LCB, NIMH)		
LAB/BRANCH Laboratory of Neurophysiology, BNP, DIR, NINDS		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 10.0	PROFESSIONAL.. 9.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Electrophysiological</u> and <u>optical recording techniques</u> are used primarily to elucidate the <u>development, differentiation</u> and cellular distribution of physiologically important properties expressed <u>in vitro</u> by vertebrate CNS neurons. Electrical studies involve direct, high-fidelity amplification of ion fluxes generated either in single cells or patches or in synaptically coupled pairs of cells maintained in monolayer culture. Optical recordings include indirect measurements of <u>membrane potential</u> or of intracellular ion concentration in small populations (50-100) of cultured cells. Principal findings this year include: 1) in the embryonic rat spinal cord voltage-dependent <u>Na⁺ channels</u> critical for propagation of activity patterns in networks emerge before <u>depolarizing GABA_A receptor-activated Cl⁻ channels</u>, which appear before depolarizing <u>glutamate</u> (kainate-type) receptor-activated <u>cation channels</u>; 2) these depolarizing conductances are <u>differentiated sequentially</u> on the <u>majority of all spinal neurons</u>; 3) activation of depolarizing conductances stimulates an <u>increase in free intracellular Ca²⁺</u> via opening of depolarization-activated Ca²⁺ channels; 4) the biophysical properties of GABA-activated Cl⁻ channels recorded in spinal cord neurons differ from those observed at the same embryonic day in olfactory neurons; 5) <u>Cl⁻ ion channels open spontaneously</u> and randomly in spinal and hippocampal neurons, thus contributing to its baseline properties and potential; 6) <u>pacemaker activity</u> driven by GABA occurs in spinal neurons; 7) Cl⁻ channel activity can be eliminated in hippocampal neurons by a gentle stream of bathing saline applied to the cell surface and by application of antagonists at GABA_A receptor-coupled Cl⁻ channels; 8) after antagonism of Cl⁻ channel activation, the stream of saline becomes ineffective, indicating that a diffusible substance, probably <u>GABA</u>, acts at the cell surface to stimulate the spontaneous Cl⁻ ion channel activities; 9) application of Zn in the bathing saline depresses spontaneous Cl⁻ ion channel activity in both spinal and hippocampal neurons; 10) in hippocampal neurons, Zn exposure leads to transient GABAergic signals whose kinetics of decay are identical to those describing the life-times of Cl⁻ channels; 11) <u>Zn transforms GABA release from random to synchronized</u>; 12) all-or-none "miniature" GABAergic transients reflecting the release of an elementary, uniform amount of GABA are summed to generate the properties of the post-synaptic signals; 13) <u>low-frequency release rates</u> generate <u>low-intensity postsynaptic signals</u> with <u>highly variable properties</u>, while <u>high-frequency release</u> creates <u>high-intensity signals</u> with more <u>precise properties</u>. </p>		
1-LNP, BNP, DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02330-15 LNP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cell Biological Studies of Developing CNS Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. L. Barker, Chief, LNP, BNP, DIR, NINDS. Others (LNP, BNP, DIR, NINDS): A.E. Schaffner, Biologist; W. Ma, Senior Staff Fellow; R. Somogyi, Senior Staff Fellow; M.O. Poulter, Visiting Fellow; D. Maric, Visiting Fellow; I. Maric, Visiting Fellow; P. Saunders, IRTA Fellow; T.N. Behar, Microbiologist; N. Hardegen, Chemist; S.V. Smith, Biologist; V. Smallwood, Bio. Lab. Technician; S. Ward, Biologist		
COOPERATING UNITS (if any) L. Hudson (Lab. Molecular & Viral Pathogenesis, NINDS); L. Mahan (LCB, NIMH); P. Laing (Dept. Immunology, University Hospital, Queens Medical Centre, Nottingham, England); J. Hickman (SAIC, Fairfax, VA); D. Stenger (Naval Research Laboratories, Washington, DC); Z. Olah, C. Lehel, W. Anderson (LCO, NCI)		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 10.0	PROFESSIONAL: 8.0	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Flow cytometry, discontinuous-gradient cell isolation, aminoacid analysis, dissociated cell culture, immunoblots, cell migration, immunochemistry, in situ hybridization and PCR methods are applied to embryonic/early postnatal rat CNS tissues to study the development, differentiation and cellular distribution of transmitter, transmitter-related enzymes and their corresponding receptors. During the past several years, we have focussed intensely on GABA, which is abundantly but transiently expressed during CNS development before it becomes restricted to fast inhibitory synapses in the adult. In FY 92 we investigated the following: 1) quantitative yield of embryonic (E) CNS cells by enzymatic digestion and mechanical dissociation resulting in complete enumeration of all cells during the embryonic period; 2) the absolute number of cells recovered from cortex, hypothalamus, midbrain, brainstem and spinal cord regions increases 50-fold over E11-13; 3) cord and brainstem cell abundance plateaus over E13-16, while other regions show parallel, 5-fold increases; 4) cord cells decline by ~90% during E15-22 along a rostrocaudal gradient while subcortical cells wane, and cortical cells increase 10-fold in number; 5) quantitative flow cytometric analysis of embryonic CNS cells exposed <u>in vivo</u> to bromodeoxyuridine (BrdU) reveals <u>anatomic gradients</u> of cellular <u>proliferation</u> and <u>differentiation</u> and their change during CNS development; 6) the percentage of BrdU⁺ cells reaches a nadir first at E15 in brainstem, at E16 in cervical spinal cord, then mid-brain/thoracic cord, followed by the rest of the CNS; 7) discontinuous <u>Percoll gradients</u> permit isolation and enumeration of all cells and their subcellular elements according to their buoyant density; 8) quantitative flow cytometric analysis of Percoll-isolated cells exposed to BrdU on E11 <u>in vivo</u> using a pulse-chase-pulse paradigm reveals characteristic patterns in cellular proliferation and differentiation correlated with cellular buoyancy; 9) sections of the embryonic CNS reveal the anatomic distribution of cells labelled in the pulse-chase-pulse protocol; 10) at E12-14, cells labelled on E11 have migrated from proliferative zones, while cells labelled 1 hr before sacrifice remain confined to these zones; 11) <u>cellular buoyancy</u> corresponds to <u>proliferation/differentiation status</u> and to <u>anatomic location in vivo</u>; 12) flow cytometric analysis of gradient-separated cells stained with molecular probes reveals characteristic patterns of physiological properties and receptor/ion-channel expressions differentiating at cellular and subcellular levels; 13) differentiating E13-15 <u>spinal neurons</u> spontaneously <u>migrate</u> through pores as well as in response to <u>gradients of GABA and NGF</u>; 14) transcripts encoding specific GABA receptor subunits appear at E12/13 in the cervical spinal cord and become colocalized during embryogenesis in anatomically distinct patterns throughout the CNS.</p>		
2-LNP, BNP, DIR		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS01659-24 LNP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synaptic Contact of Retinal Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Lasansky, Unit Chief, LNP, BNP, DIR, NINDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Unit on Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The action of synaptic blocking agents on the chloride-dependent ON-OFF responses to central illumination of depolarizing bipolar cells was investigated in salamander retinal slices by means of whole cell recordings. Strychnine sulfate (10-50 μ M) and bicuculline methobromide (50-500 μ M) did not have any effect. Picrotoxin (50-100 μ M) transiently decreased the current amplitude to 40-50%, but within approximately one minute the current increased again to stabilize at 60-70% of control amplitude. Except for the incomplete block by picrotoxin, these results appear to fit a recent description of a third class of GABA receptor.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS 01659-09 LNP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function in Retinal Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Ralph Nelson Unit Chief LNP, NINDS
 Others: Michael A. Freed Staff Fellow LNP, NINDS

COOPERATING UNITS (if any)

Physiology, University of Vienna, Austria (Renate Pflug)
 Physiology, University of Utah School of Medicine, Salt Lake City (Helga Kolb)
 Mathematics, Arizona State University, Tempe (Steven Baer)
 Psychology, Queens College, City University of New York (Thomas Frumkes)
 Anatomy, University of Pennsylvania, Philadelphia (Peter Sterling, Robert G. Smith)

LAB/BRANCH

Laboratory of Neurophysiology, BNP, DIR, NINDS

SECTION

Neural Circuitry Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neural organization and neural interactions in mammalian retinas are investigated using intracellular electrophysiology, electron microscopy, and pharmacology. GABAergic effects have been investigated on horizontal, amacrine, and ganglion cells of cat retina. In all these cells GABA_A antagonists increase the speeds and amplitudes of transient responses while GABA_A agonists slow such responses. Actions on horizontal cells appear mediated by a receptor insensitive to bicuculline methyl halides. The influence of selective D1 and D2 agonists on horizontal-cell responses has been observed in cat and rabbit retinas. The D2 agonist LY171555 selectively enhances rod signal components at low stimulus intensities while leaving unaffected cone signal components seen at high intensities. The D1 agonist SKF38393 also enhances rod signals at low concentrations, but at higher concentrations, suppresses cone signals in addition. These effects point to multifaceted regulation of outer-plexiform layer circuitry by both GABA and dopamine receptors.

Biophysical properties of cat ON-β ganglion cells were investigated by extrinsic current injection. Two response components were resolved: a short latency excitatory component and a longer latency, wider field inhibitory component. These combine to produce the signature transient-sustained depolarization seen with wide-field stimuli. Type b₁ bipolars may provide excitation, while other bipolar or amacrine cells provide inhibition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02767-05 LNP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.) Image Processing and Analysis of Cellular Structures		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: T.G. Smith, Jr., Unit Chief, LNP, BNP, DIR, NINDS. Others (LNP, BNP, DIR, NINDS): Anne E. Schaffner, Biologist, T.N. Behar, Technician		
COOPERATING UNITS (if any) G.D. Lange, (ICS, NINDS), W.B. Marks (LNLG, NINDS), E.A. Neale (LDB, NICHD); W.H. Sheriff, Jr. (IACS, NINDS); Seth R. Goldstein, (BEIP, NCRR); Robert Porter (Monash University, Australia); Andreas Reichenbach (Leipzig University, Germany)		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION Unit on Sensory Physiology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 2.7	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have continued to employ the concepts of Mandelbrot's fractal geometry to the quantitative studies of <u>central nervous system neurons</u>, and other cell types grown in tissue culture or from whole animals. We do this by employing image processing techniques to measure the <u>fractal dimension (FD)</u>, which is a measure of the <u>complexity of the structure under investigation</u>. In particular, the FD relates to the <u>degree of branching</u> (e.g., of dendrites), the <u>ruggedness of borders</u>, and the <u>degree of space-filling</u> of the object of interest. </p> <p> We have undertaken, in separate studies, how the fractal dimension changes during the <u>differentiation and growth of glial and neuronal cells in tissue culture</u>. We have found that <u>optic nerve-derived oligodendrocytes</u> differentiate faster and to a greater extent than do <u>nerve-derived astrocytes</u>. A subsequent study has found that <u>nerve-derived glia</u> also differentiate faster and to a greater extent than do <u>brain-derived glia</u>. Interestingly, the rates of differentiation as measured by the FD can be described by a single time constant. The work on <u>cultured spinal neurons</u> shows that they differentiate in a similarly simple fashion. We have proposed that the FD is a <u>useful, quantitative measure of morphological differentiation</u>. </p> <p> We have begun studies of the development of the internal and surface structures of <u>cultured rat hippocampal neurons with fluorescence and confocal microscopy</u> in order to localize the position of <u>GABA</u> during growth and differentiation. </p>		

ANNUAL REPORT
October 1, 1991 through September 30, 1992
Laboratory of Molecular and Cellular Neurobiology
Basic Neurosciences Program, DIR

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ANNUAL REPORT

October 1, 1991 through September 30, 1992

Laboratory of Molecular and Cellular Neurobiology National Institute of Neurological Disorders and Stroke

Richard H. Quarles, Ph.D., Chief (Acting)

The Laboratory of Molecular and Cellular Neurobiology (LMCN) now consists of the Membrane Biochemistry Section (MBS) headed by Dr. Peter Fishman and the Myelin and Brain Development Section (MBDS) headed by Dr. Richard Quarles. These two Sections have closely related research programs with a common interest in membrane biochemistry, cell surface glycoconjugates and the use of cultured cells to investigate biochemical aspects of neurobiology. In addition, the Neural Transplantation Unit (NTU), headed by Dr. John Commissiong and also located in the Park Building, was transferred into LMCN from the Clinical Neuroscience Branch during this fiscal year. The research program of NTU, emphasizing neural regeneration and the effects of growth factors, effectively complements the research in MBS and MBDS. Monthly meetings in LMCN are continuing in which scientists from the two Sections and new Unit present their recent results to the whole Laboratory as a step toward promoting communication and collaboration. Also, research involving molecular biology techniques in the Laboratory continues to grow as a result of the effective recruiting and hiring of new scientists with experience in this area. Lastly, LMCN is in the final stages of planning for the move to the new Child Health and Neuroscience Building (Bldg. 49) in the next fiscal year. It is expected that this move back to the main campus with greater opportunities for communication and collaboration with other laboratories and branches will provide an effective boost to both the basic and clinically-related aspects of neuroscience research in LMCN.

Myelin and Brain Development Section

Research in the Section on Myelin and Brain Development is divided into four related projects. The project entitled "Glycoproteins of Myelin in Development and Disease," has been in existence for over 20 years and emphasizes the myelin-associated glycoprotein (MAG). However, other myelin proteins and lipids are also studied with the ultimate objectives of understanding molecular mechanisms of myelin formation and breakdown. During this fiscal year, a new Demyelinating Disorders Unit headed by Dr. Johanna Moller was established in MBDS and will be the focus of research on disorders of myelin in the CNS including multiple sclerosis (MS) and the leukodystrophies. In relation to the formation of this Unit, a new project entitled "Disorders of CNS Myelin" is being established this year with Dr. Moller as the principal investigator, and will henceforth include many aspects of the research on demyelinating diseases of the CNS that were previously covered in "Glycoproteins of Myelin in Development and Disease." Another project that emphasizes demyelinating diseases of the PNS is entitled "Antibodies to Glycoconjugates in Neurological Diseases." This area of research first developed as a consequence of the discovery that monoclonal IgM antibodies to MAG in patients with neuropathy cross reacted with other glycoproteins and glycolipids. This research grew to include, not only the occurrence and pathogenic significance of anti-MAG antibodies, but also antibodies to gangliosides and other sphingoglycolipids in patients with peripheral neuropathies. Also included in this

project is a growing area of research on the biosynthesis and function of glycolipids during PNS myelination with the ultimate objectives of determining how the anti-glycolipid antibodies associated with neuropathy may perturb these functions. Finally, when it became apparent that a significant aspect of the neuropathology in acquired immunodeficiency syndrome (AIDS) involves white matter, research was undertaken to elucidate molecular mechanisms involved in the neurological aspects of this disease. This project is entitled "Molecular and Immunological Aspects of Myelin Abnormalities in Neuro-AIDS".

1. Structure and Function of the Myelin-Associated Glycoprotein (MAG). The myelin-associated glycoprotein (MAG) has a single membrane-spanning domain separating its C-terminal tail from a heavily glycosylated N-terminus that is composed of 5 domains related in amino acid sequence to each other and to other members of the immunoglobulin superfamily such as the neural cell adhesion molecule (N-CAM). These extracellular immunoglobulin-like domains must mediate the function of MAG in glia-axon interactions, possibly by specifically interacting with another member of the superfamily on the axolemma. Homophilic MAG binding may be involved in the interactions of adjacent Schwann cell membranes in incisures, lateral loops and mesaxons. MAG occurs in two developmentally regulated isoforms differing in the lengths of their C-terminal domains and arising by alternative splicing of the primary mRNA transcript. Both CNS and PNS MAG are phosphorylated on their cytoplasmic domains, but the sites of phosphorylation are different. Regulation of expression of the two forms of MAG as well as phosphorylation of the cytoplasmic domains may modulate interactions with cytoskeletal components. Overall, the MAG molecule seems well suited to mediate interactions between intracellular cytoskeletal elements and adjacent extracellular membrane surfaces. In this manner, MAG could play a key role in transmitting the chemomechanical forces involved in generating the spiraled myelin sheaths.

MAG has 8 extracellular sites for N-linked glycosylation, and detailed investigation of the oligosaccharides are underway utilizing the Dionex BioLC Carbohydrate System. This procedure has demonstrated that the oligosaccharides of MAG are heterogeneous and many are highly negatively charged due both to sialic acid and sulfate groups. A prominent neutral, high mannose oligosaccharide of MAG has been isolated and characterized by glycosidase treatment and fast atom bombardment-mass spectrometry. Its structure is $\text{Man}\alpha 1-6(\text{Man}\alpha 1-3)\text{Man}\alpha 1-6(\text{Man}\alpha 1-3)\text{Man}\beta 1-4\text{GlcNAc}\beta 1-4\text{GlcNAc}$. Changes in oligosaccharide structure of MAG may modulate the functioning of MAG in membrane-membrane interactions. MAG is abnormally glycosylated in the dysmyelinating quaking mutant which may lead to some of the myelin structural abnormalities in this mutant.

The HNK-1/L2 carbohydrate epitope is expressed on MAG and appears to be characteristic of adhesion proteins in the nervous system including N-CAM. It has been known for some time that several 19-28 kDa glycoproteins in PNS myelin of some species such as cats and humans express high levels of this carbohydrate structure. This year we demonstrated that the glycoprotein of cat peripheral myelin expressing the largest amount of this epitope is the recently characterized PMP-22, suggesting that this protein could function in cell-cell interactions.

Molecular biological techniques are increasingly being applied to our research on the function of glycoproteins in myelin formation. As a step toward understanding

the control of MAG expression in humans, several cosmids from human chromosome 19 have been obtained and sequencing of the human MAG gene is underway. We are also sequencing a newly identified human adhesion protein in the Ig superfamily which is likely to be a human homologue of the avian Nr-CAM.

An investigation of MAG in cultured oligodendrocytes and Schwann cells is under way with the ultimate objectives of investigating factors that control its expression and probing its function as an adhesion molecule. The experiments on oligodendrocytes are currently being pursued with oligodendrocytes purified from primary cultures of immature rat brain by the "shake off" procedure. MAG is actively phosphorylated in the oligodendrocytes, and the phosphorylation is catalyzed at least in part by protein kinase C (PKC) and a calcium-activated kinase. Treatment of the rat oligodendrocyte cultures, which consist of O2A progenitors as well as more mature oligodendrocytes, with exogenous GM3 ganglioside (NeuNAc α 2-3Gal β 1-4Glc β 1-1'ceramide) promotes their differentiation in a manner similar to that described last year for mature bovine oligodendrocytes. As was the case for mature bovine oligodendrocytes, the effect is relatively specific for GM3 ganglioside and was not produced by GM1, GM2, GD3 or GD1a. Immunostaining of the treated cells as well as biochemical experiments indicated that the exogenous GM3 promotes differentiation of the oligodendrocytes in the direction of myelin formation. These findings raise the possibility that exogenous ganglioside may stimulate remyelination *in vivo* during the regeneration of injured neural tissues similarly to their established effects on neuronal regeneration.

Since cultured Schwann cells do not constitutively express MAG, we are now studying some immortalized Schwann cell lines that express remarkably high amounts of MAG. Three immortalized Schwann cell lines are currently being maintained: line #1 expresses the highest level of MAG comparable to that in adult sciatic nerve; line #2 expresses a somewhat lower level of MAG; and line #3 does not express detectable MAG. The amount of MAG expressed by these three cell lines is inversely proportional to their rates of growth and directly proportional to the number of processes on the cells. The phosphorylation of MAG in the cells is primarily on serine residues as is the case for peripheral nerve, and is mediated, in part, by PKC. The overall protein compositions of the three lines observed on Coomassie Blue stained gels are very similar, but the MAG-expressing lines generally express higher levels of glycoproteins. The two MAG-expressing lines also express low amounts of P₀ glycoprotein, N-CAM, L1 glycoprotein, galactocerebroside and sulfatide, but no myelin basic protein (MBP). When co-cultured with dorsal root ganglion neurons, the MAG-expressing cell lines slowly aligned themselves with the neurons over several days, whereas the MAG-negative line did not. Also, the line expressing the highest amount of MAG bound larger amounts of purified axolemma than the MAG-negative line. However, conditions have not yet been found in which any of the lines will myelinate DRG neurons in culture. It is expected that these immortalized Schwann cells will continue to be useful for studying the cell biology of MAG and its function in cell-cell interactions.

2. Multiple Sclerosis (MS) and Other Disorders of CNS Myelin. Previous studies had demonstrated a greater loss of MAG than other myelin proteins at the periphery of MS plaques, suggesting an important role for MAG in the molecular pathogenesis of this disease. Experiments are in progress to further define the molecular mechanism responsible for the selective MAG loss which appears to involve a myelin-associated, calcium-activated protease.

The studies on postmortem brains from Niemann-Pick type C disease were completed this year. The biochemical results showed a myelin deficiency similar to that previously shown in the cholesterol storage disorder (CSD) murine model with a similar metabolic defect, but the myelin deficit in the human disease was relatively mild and varied considerably from region-to-region and between the two patients examined.

MAG and other myelin components continue to be examined in animal models to gain insight about their function in development and their roles in demyelinating disorders. This year we have investigated a new neurological rodent mutant in collaboration with Dr. Ian Duncan of the University of Wisconsin. This *taiep* mutant arose as a spontaneous mutation in a colony of Sprague-Dawley rats. The affected rats develop a progressive neurological syndrome characterized by trembling, ataxia, immobility episodes, epilepsy and paralysis (hence the acronym *taiep*). Histological studies revealed a progressive ongoing loss of myelin beginning at a few months of age that is associated with a dramatic accumulation of microtubules in oligodendrocytes. Biochemical results were consistent with this, showing a yield of myelin that was about 50% of normal at 2 months of age, and then decreased until it was only a few percent of normal at 12 months. All of the well-characterized myelin proteins were present, but the results for MAG were particularly interesting. Unlike most hypomyelinating mutants that have been studied in which MBP and proteolipid protein are decreased more than MAG (apparently due to a greater deficiency of compact myelin than of MAG-containing periaxonal membranes), MAG was decreased substantially more than the proteins of compact myelin in the *taiep* mutant. Furthermore, only the larger immature isoform of MAG was detected in the relatively mature mutants. It may be that there is a defect in microtubule-associated transport of myelin proteins in the mutant oligodendrocytes, and MAG could be preferentially affected because it is the most distally located protein from the cell body in the periaxonal membranes. Younger *taiep* mutants are now being examined to further understand the defect in this interesting hypo- and demyelinating mutant.

3. Antibodies to Glycolipids in Peripheral Neuropathies. This area of investigation began with the demonstration of monoclonal anti-MAG antibodies in patients with polyneuropathies occurring in association with IgM gammopathy (paraproteinemia). It was subsequently demonstrated that these anti-MAG antibodies cross-reacted with the sphingoglycolipid, sulfate-3-glucuronyl paragloboside (SGPG). Further studies showed that MAG/SGPG-negative monoclonal IgM antibodies in other neuropathy patients frequently reacted with various ganglioside antigens. Overall, about 80% of patients with neuropathy occurring in association with IgM paraproteinemia have a monoclonal antibody that reacts with SGPG or a ganglioside antigen, suggesting that glycolipids may be important target antigens in this type of neuropathy.

In the current year, we completed our studies on a patient with sensory neuropathy and a monoclonal IgM antibody reacting with GD1b ganglioside as well as other gangliosides with disialosyl groups. This finding plus others in the literature suggest that patients with predominantly sensory neuropathy and anti-GD1b antibodies may represent a distinct subset of the paraproteinemic neuropathies. In addition, we have identified two other neuropathy patients with monoclonal anti-ganglioside

antibodies. One patient had IgA reacting with the major LM1 ganglioside of peripheral nerve myelin and a higher homologue of this ganglioside. This patient is particularly interesting, since little is known about antigenic targets in patients with IgA gammopathy and neuropathy. Another patient had IgM reactive with multiple gangliosides, but with a tendency to bind most strongly to monosialogangliosides.

Little is known about the molecular mechanisms by which monoclonal antibodies to acidic glycolipids in patients with demyelinating neuropathy (or polyclonal antibodies to glycolipids in the inflammatory neuropathies described in previous reports) exert their pathogenic effects. In order to probe the function of acidic glycolipids in myelination and to understand mechanisms by which the human anti-glycolipid antibodies may perturb function, we have begun an investigation of gangliosides in differentiating Schwann cells. In comparison to Schwann cells cultured in the absence of neurons in serum-containing medium on polylysine, culturing the Schwann cells on a basement membrane substratum (Matrigel) resulted in increased incorporation of radioactive galactose into gangliosides (predominantly GM3) but had no effect on incorporation into the galactosphingo glycolipids characteristic of myelin. Thus, although the basement membrane is well known to be required for myelination, its presence in the absence of neurons drives Schwann cell lipid metabolism toward gangliosides rather than toward the galactosphingolipids enriched in myelin. An important priority for the future is to evaluate the capacity of anti-ganglioside antibodies to cause neural damage or interfere with normal function in appropriate animal models or tissue culture systems.

4. Biochemical Neuropathology in Acquired Immunodeficiency Syndrome (AIDS).

Relevant areas of our research described in the preceding sections are being extended in an attempt to determine the molecular basis for some of the abnormalities of myelin occurring in neuro-AIDS, including myelin pallor, vacuolar myelopathy and multifocal demyelination in the CNS as well as demyelinating peripheral neuropathy. Postmortem CNS tissues from AIDS patients are being analyzed for quantitative and qualitative alterations of myelin proteins. The biochemical results are being correlated with histological and immunocytochemical observations made by our collaborators at Johns Hopkins University on adjacent sections of tissue. So far, the immunocytochemical staining of white matter of AIDS CNS has not suggested substantial loss of myelin proteins in areas where there is prominent myelin pallor, but a definitive conclusion on this must await the results of our quantitative biochemical studies currently in progress on AIDS cases with dementia in which myelin abnormalities are most common. It has been suggested that some of the myelin abnormalities in AIDS, and other neurological diseases caused by retroviruses, may be related to autoimmune phenomena. However, Western blotting experiments done this year have not revealed antibodies to proteins of CNS or PNS myelin in sera from rabbits with HTLV-1 or monkeys infected with HIV.

Neural Transplantation Unit

The Neural Transplantation Unit does research on reinnervation and functional recovery following spinal cord injury in a rat model. When a complete cut of the spinal cord is made at the midthoracic level in a neonatal rat at the 7th postnatal day (PN7) or earlier, the hindlimbs spontaneously recover the ability to make rhythmic steps, enabling the animal to walk, although with an ataxic gait. Physiological studies indicate that recovery is due, at least in part, to Gp1a fibres making functional

contact with alpha motor neurons and an enlarged receptive field for motor neurons in the triceps surae muscles. Recovery is minimal or absent when cordotomy is done at PN14 or later, but can be improved to a level comparable to the PN7 rats by treatment with GM1 ganglioside or injection into the spinal cord of a suspension of embryonic ventral mesencephalic cells that are 20% tyrosine hydroxylase positive. These results demonstrate that the lumbosacral central pattern generator exhibits plasticity and is capable of functional modification, and there is a large body of neurophysiological data indicating that it can be modified by catecholamines. However, one of the principal limitations of the effectiveness of treatment by transplantation appears to be neuronal death. Exciting preliminary results in tissue culture indicate that survival and dendritic proliferation of tyrosine-positive neurons can be promoted by a neurotrophic factor elaborated by astrocytes of the ventral mesencephalon. This neurotrophic factor appears to be specific for rescuing dopaminergic neurons, and future research in the Unit will emphasize its isolation, characterization and effects on functional recovery in transplantation experiments.

Membrane Biochemistry Section

The **Membrane Biochemistry Section** is actively investigating the structure, biosynthesis and regulation of cell membrane components involved in various recognition phenomena and in cellular signaling. These include complex glycoconjugates such as gangliosides and the receptor-coupled adenylyl cyclase which mediate the cellular responses to various hormones, neurotransmitters and growth factors as well as pathological toxins and viruses. Most of our studies involve the use of cultured cell lines which express these components and respond to physiological and environmental signals. We have made three major findings this year.

1. Orientation of Cholera Toxin and Its Intracellular Processing. We had shown that ganglioside GM_1 is the only endogenous cell surface receptor for cholera toxin (CT), the active agent in the disease cholera. CT intoxicates target cells by ADP-ribosylation of the α subunit of the stimulatory G protein, G_s , of adenylyl cyclase which results in its persistent activation. The toxin consists of a pentameric B subunit which binds to GM_1 on the cell surface, and an A subunit which consists of A_2 and A_1 peptides linked by a disulfide bond. Reduction of CT-A releases the A_1 peptide which is the ADP-ribosyltransferase. The orientation of CT when it binds, the pathway by which the A subunit enters cells and is reduced to A_1 , and how the latter gains access to G_{sa} have not yet been established. Two major models have developed over the years. In the first, after CT binds to the cell membrane, the A subunit unfolds, penetrates across the membrane, and is reduced to release the A_1 peptide, which refolds and ADP-ribosylates G_{sa} . In the second, the holotoxin is internalized through noncoated invaginations in the cell membrane which form noncoated vesicles. The latter enter the membrane traffic pattern of the central vacuolar system of the cell and are routed and processed in such a way that A_1 is released, escapes from the luminal compartment into the cytosol, and reaches G_{sa} . The recently published three-dimensional crystal structure of *E. coli* heat-labile enterotoxin which causes travelers' diarrhea shows that the toxin is asymmetric and can bind in two different orientations: 1) with the B subunit held close to the membrane by GM_1 oligosaccharides and A_1 facing away; or 2) with the B subunit tethered above the membrane by GM_1 oligosaccharides and A_1 directed into the membrane. These two orientations have implications for the two models discussed above.

In order to identify the orientation of CT when it binds to the cell surface, we developed a novel technique which involves sequentially assembling holotoxin on the cell surface from its subunits. For these experiments, we used human neurotumor SK-N-MC and human intestinal Caco-2-cells. The latter grow in culture as differentiated enterocytes, the natural target cell for CT. First, we incubated cells at 4°C with purified CT-B, washed the cells free of any unbound CT-B, and then exposed them to purified CT-A. The cells were washed again, warmed up to 37°C and assayed for cyclic AMP accumulation. Whereas cells exposed to the individual subunits were unresponsive, cells sequentially exposed to the B and A subunits produced large amounts of cyclic AMP. Reversing the order of subunit addition resulted in no response. Our results are consistent with cell surface bound CT being oriented with its A subunit facing away from the membrane. Using antibodies raised against CT-B and the A₁ peptide, we confirmed that the holotoxin was sequentially assembled on the cells from the addition of B and A subunits. In this regard, cells exposed to CT bound similar amounts of the two types of antibodies, supporting the proposed orientation. If CT-A faced into the membrane, it would be buried under the pentameric B subunit and inaccessible to the anti-A₁ antibodies. Finally, we used the two types of antibodies to follow the disappearance of toxin subunits from the cell surface. The cells were exposed to CT at 4°C, washed and warmed up to 37°C. At different times, the cells were assayed for cell surface immunoreactivity. Both subunits disappeared from the cell surface with time. Significant disappearance occurred prior to any increase in cyclic AMP. We confirmed that disappearance of CT from the cell surface was not due to its dissociation into the media, but due to its internalization.

To identify the steps involved in the intracellular processing and activation of CT, we explored the effects of several known inhibitors. Chloroquine and monensin inhibit receptor-mediated endocytosis through coated pits by raising the pH of the endosome. The third drug we used was brefeldin A (BFA) which blocks the export of proteins from the Golgi apparatus, causes a rapid disassembly of the Golgi as a distinct morphological compartment, and induces the redistribution of Golgi resident components into the endoplasmic reticulum (ER). We tested the effects of these drugs on human SK-N-MC neurotumor and Caco-2 intestinal cells. Concentrations of chloroquine and monensin which inhibited the degradation of ¹²⁵I-CT, had no effect on the response of these cells to CT. BFA was a potent blocker of CT stimulation of both cells with an IC₅₀ of 30 ng/ml (0.1 μM). BFA acted very rapidly and blocked stimulation even when added to the cells at the same time as CT. This was important as there is a characteristic lag period between toxin binding and activation of adenylyl cyclase. Presumably during the lag, CT is internalized and undergoes processing to generate the A₁ peptide which then reaches G_{sα} and ADP-ribosylates it. BFA had no effect on CT internalization but completely blocked the generation of A₁ by intact cells. Based on what is known about the effects of BFA, its ability to block CT action is most likely due to one of two possibilities: either CT or CT-A must pass through a functioning Golgi apparatus in order to be converted to A₁; or some factor required for CT activation must be processed through the Golgi. Although we are unable at present to distinguish between these two possibilities, we recently realized that the amino acid sequence KDEL is present on the C-terminus of the A₂ peptide. This sequence has been identified as an ER retention signal which allows resident ER luminal proteins to be retrieved from more distal compartments in the exocytosis pathway. It may be that once the A subunit dissociates from the B subunit, it is directed toward the ER where it undergoes reduction to release the A₁ peptide. The latter, free of A₂ and its ER retention signal, follows the anterograde pathway from ER to Golgi and eventually to the plasma membrane.

Finally, it is interesting to compare the effects of the three drugs on two other ADP-ribosylating bacterial toxins, diphtheria toxin and *Pseudomonas* exotoxin, which inhibit protein synthesis by ADP-ribosylation of elongation factor 2. Both toxins bind to receptors localized in coated pits, enter the cell through receptor-mediated endocytosis, undergo proteolytic processing and reduction, and release their active A fragments into the cytosol. As predicted, both chloroquine and monensin are potent blockers of these two toxins. Whereas BFA has no effect on diphtheria toxin, it does protect cells from *Pseudomonas* exotoxin. We have confirmed the latter; and in our hands, BFA inhibited the exotoxin at the same concentrations which inhibited cholera toxin. The C-terminus of *Pseudomonas* exotoxin has the sequence REDLK, an ER retention signal. Even though CT and the exotoxin enter the cells by different mechanisms, the two toxins may share some common sites in their intracellular processing, particularly those involving the Golgi and ER, and their interconnecting pathways. By contrast, it is believed that diphtheria toxin is processed in endosomes from which its A fragment is released into the cytosol. Thus, brefeldin A may be a useful probe for delineating the intracellular processing of CT.

2. Differences in Regulation of Human β -Adrenergic Receptors. Previously, we reported that the desensitization of human β_1 -adrenergic receptors endogenously expressed in SK-N-MC neurotumor cells was different than that of the human β_2 -adrenergic receptor, either endogenously expressed in human cell lines or stably expressed in transfected hamster and mouse cells. Both receptors are susceptible to the action of the cyclic AMP-dependent protein kinase as evidenced by a rightward shift in the dose response for agonist stimulation of adenylyl cyclase activity in desensitized cells. Only the β_2 receptor, however, is desensitized by the β -adrenergic receptor-specific kinase as seen by a reduction in maximum stimulation. In addition to desensitization, exposure of cells to agonists results in sequestration and down-regulation of receptors. These are operational definitions as the exact location and fate of the receptors has not been established. Sequestered β receptors are inaccessible to hydrophilic ligands, but can be detected by the more hydrophobic ligands. Down-regulated β receptors are not detected by any ligands. When SK-N-MC cells are exposed to isoproterenol, their β_1 receptors undergo sequestration but are resistant to down-regulation. By comparison, similar studies with human A431 cells, which express endogenous β_2 receptors, showed that their receptors underwent both sequestration and down-regulation.

In order to extend these studies, we have constructed an eukaryotic vector in which we inserted a cDNA containing the entire coding region of either the human β_1 AR or β_2 AR. We transfected two different hamster cell lines with the vector, selected resistant cells, and subcloned them to obtain stable cell lines expressing different levels of either receptor subtype. We also obtained from other laboratories stably transfected cell lines expressing either β_1 or β_2 receptors. Although we have not yet analyzed all of these cell lines for agonist-mediated sequestration, down-regulation and desensitization, our initial results are promising. We found that human β_2 receptors were sequestered faster and more extensively than human β_1 receptors. Down-regulation was much more complicated and appeared to be affected by cell type. In the case of desensitization, the two β_1 receptor-expressing cell lines analyzed so far were resistant to acute agonist exposure whereas the one β_2 receptor-expressing cell line exhibited a 43% reduction in responsiveness.

These observations support our hypothesis that there are differences in the regulation of human β_1 - and β_2 -adrenergic receptors. We believe that these differences may be related to structural variations between the two receptor subtypes. Both belong to the family of G protein-linked receptors with seven

transmembrane-spanning regions and are highly homologous in these transmembrane regions. There are significant differences in the amino acid sequences of their C-termini; this region of the receptor is cytoplasmic and accessible to protein kinases and other potential regulator factors. The $\beta 2$ receptor has eleven potential phosphorylation sites for the receptor-specific kinase in its C-terminus where as the $\beta 1$ receptor has only eight such sites. We believe that these differences may account for the differences in their susceptibility to desensitization by the receptor-specific protein kinase.

3. Role of Subunit Dissociation in Interaction of G_s with Adenylyl Cyclase. It is well established that many receptors for hormones and neurotransmitters are coupled to their effector systems through guanine nucleotide binding proteins (G proteins). Thus, stimulatory receptors activate adenylyl cyclase through G_s and inhibitory receptors inhibit adenylyl cyclase through G_i . These G proteins are heterotrimers with common $\beta\gamma$ and distinct α subunits. The current model envisions that agonist-bound receptors activate their respective G proteins by promoting the exchange of GDP with GTP which results in dissociation of the heterotrimers and release of the α subunits containing bound GTP. The activated α subunit then stimulates the effector. In time, the bound GTP is hydrolyzed to GDP by the intrinsic GTPase activity of the α subunit which then reassociates with the $\beta\gamma$ subunits to complete the cycle. Most of the evidence for this model is based on the behavior of purified components in solution. Previous studies by us and others have raised the possibility that this subunit dissociation model may not be applicable in biological membranes.

In order to pursue this problem, we have examined the activation and dissociation of purified G_s by the nonhydrolyzable GTP analogue, GTP γ S, and MgCl₂. We have found that the G_s subunit dissociation can occur in the absence as well as the presence of GTP γ S; thus subunit dissociation can occur without G_s activation. By incubating G_s with GTP γ S and low concentration of MgCl₂ (2 mM), we were able to completely and irreversibly activate the protein without causing detectable subunit dissociation. Higher concentrations of MgCl₂ (20 mM) induced subunit dissociation. This process was linear with time, and required more than 6 hours to be completed. G_s activation in the presence of 20 mM MgCl₂ was nonlinear with time, and was 85% complete by 2 hours when less than 30% was dissociated. These results suggest that activation of G_s can occur in advance of subunit dissociation, and that the latter may not be required for G_s activation. Finally, they have important implications for understanding the mechanism of activation of adenylyl cyclase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01NS01808-23LMCN
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Glycoproteins of Myelin in Development and Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Richard H. Quarles, Ph.D. Others: Sung Hye Yim, Ph.D. John Prince, Ph.D. Judy Small, Ph.D. Zbigniew Bartoszewicz, Ph.D. Kenichi Toda, M.D.	Section Chief, LMCN, NINDS Special Expert, LMCN, NINDS Sr. Staff Fell., LMCN, NINDS Sr. Staff Fell., LMCN, NINDS Vist. Assoc., LMCN, NINDS Vist. Fellow, LMCN, NINDS	Carl Lauter, Chemist Jeffrey Hammer, Biologist
COOPERATING UNITS (if any) Department of Neurology, Johns Hopkins University, Baltimore, Maryland; Laboratory of Biophysical Chemistry, National Heart Lung and Blood Institute		
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology		
SECTION Myelin and Brain Development Section		
INSTITUTE AND LOCATION Park Bldg, Rm. 425, NINDS, NIH, Bethesda, MD. 20892		
TOTAL STAFF YEARS: <div style="text-align: center; font-size: 1.2em;">5.7</div>	PROFESSIONAL: <div style="text-align: center; font-size: 1.2em;">4.5</div>	OTHER: <div style="text-align: center; font-size: 1.2em;">1.2</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%; text-align: center;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The <u>myelin-associated glycoprotein (MAG)</u> is localized in the <u>periaxonal membranes</u> of PNS and CNS myelin sheaths where it appears to be involved in <u>glia-axon interactions</u>. MAG is a member of the <u>immunoglobulin gene superfamily</u> along with other <u>neural adhesion proteins</u>, and <u>alternative splicing</u> of its mRNA generates two <u>developmentally regulated isoforms</u> with differing C-terminal tails. The extracellular domains of the two forms of MAG are identical with 5 immunoglobulin-like domains and 8 potential sites for N-linked <u>glycosylation</u>. The carbohydrate consists of a mixture of <u>oligosaccharides</u>, many of which are sialylated and sulfated, and which are currently being isolated and characterized. A prominent neutral oligosaccharide of rat MAG has been identified as Mana1-6(Mana1-3)Mana1-6(Mana1-3)Manβ1-4GlcNAcβ1-4GlcNAc. It was also demonstrated that the <u>adhesion-related HNK-1/L2</u> carbohydrate epitope expressed on MAG is expressed on the recently cloned <u>PMP-22</u> of peripheral myelin suggesting that PMP-22 may function in cell-cell interactions. The expression of MAG in <u>cultured oligodendrocytes</u> and <u>Schwann cells</u> is being studied with the ultimate objectives of identifying factors that control its synthesis and probing its function in cell-cell interactions. MAG is phosphorylated in cultured oligodendrocytes as it is <u>in vivo</u>, and the <u>phosphorylation</u> appears to be catalyzed at least in part by <u>protein kinase C</u> and a <u>calcium-activated kinase</u>. Addition of exogenous <u>GM3 ganglioside</u> to the culture media stimulates the formation of oligodendroglial processes in a manner reminiscent of the well established neuritogenic effects of gangliosides. Biochemical analyses of the GM3-treated oligodendrocytes demonstrate that the ganglioside promotes differentiation in the direction of myelination. Although cultured primary <u>Schwann cells</u> do not normally express MAG in the absence of neurons, some <u>immortalized Schwann cell lines</u> generated in our laboratory express remarkably high levels of MAG, and the post-translational <u>glycosylation</u>, <u>sulfation</u> and <u>phosphorylation</u> in these cells is similar to that <u>in vivo</u>. The amount of MAG expressed by these cell lines is inversely proportional to their rates of growth and directly proportional to the number of processes on the cells. The phosphorylation of MAG is primarily on serine residues, and it is mediated in part by protein kinase C. These cell lines expressing high levels of MAG are being used to investigate the cell biology and function of this <u>glycoprotein</u>. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02786-04LMCN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antibodies to Glycoconjugates in Neurological Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Richard H. Quarles, Ph.D.	Section Chief	MBDS, LMCN, NINDS
OTHERS:	Robert Farrer, Ph.D.	Staff Fellow	MBDS, LMCN, NINDS
	Carl Lauter	Chemist	MBDS, LMCN, NINDS
	Jeffrey Hammer	Biologist	MBDS, LMCN, NINDS

COOPERATING UNITS (if any)

Medical Neurology Branch, NINDS

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Myelin and Brain Development Section

INSTITUTE AND LOCATION

Park Building, Rm. 425, NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF YEARS:

1.6

PROFESSIONAL:

1.2

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This area of investigation began with the demonstration of monoclonal anti-MAG antibodies in patients with mixed sensory-motor polyneuropathies occurring in association with IgM gammopathy (paraproteinemia). It was subsequently demonstrated that these anti-MAG antibodies were all directed toward carbohydrate epitopes in MAG and cross-reacted with 19 to 28 kD glycoproteins of PNS myelin and a sphingoglycolipid, sulfate-3-glucuronyl paragloboside (SGPG). Monoclonal antibodies that are MAG/SGPG-negative in patients with IgM gammopathy and neuropathy frequently react with ganglioside antigens in nerve. Previous results from our laboratory and others had shown a strong correlation of high titer, monoclonal and polyclonal antibodies to G_{M1}-ganglioside with motor neuropathies. In the current year, we completed studies on a patient with a predominantly sensory neuropathy and a monoclonal IgM antibody that binds strongly to G_{D1b}-ganglioside and some other minor gangliosides that also contain a disialosyl group. Interestingly, two other patients with sensory neuropathy and antibodies of similar specificity have been reported, suggesting that antibodies of this general specificity may play a role in the pathogenesis of sensory neuropathies. In addition, we identified other neuropathy patients with monoclonal anti-glycolipid antibodies, including one with monoclonal IgA reacting with the major L_{M1} ganglioside of peripheral nerve myelin. Little is known about the molecular mechanisms by which antibodies to acidic glycolipids in patients with demyelinating neuropathy exert their pathogenic effects. In order to probe the function of acidic glycolipids in myelination and to understand mechanisms by which the human anti-glycolipid antibodies may perturb function, we have begun an investigation of gangliosides in differentiating Schwann cells. In comparison to Schwann cells cultured in the absence of neurons in serum-containing medium on polylysine, culturing the Schwann cells on a basement membrane substratum (Matrigel) resulted in increased incorporation of radioactive galactose into gangliosides (predominantly G_{M3}) and had no effect on incorporation into the galactosphingoglycolipids characteristic of myelin. Thus, although basement membrane is well known to be required for myelination, its presence in the absence of neurons drives Schwann cell lipid metabolism toward gangliosides rather than toward the galactosphingolipids enriched in myelin.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS02805-03LMCN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Immunological Aspects of Myelin Abnormalities in Neuro-AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Richard H. Quarles, Ph.D.	Section Chief	MBDS, LMCN, NINDS
OTHERS:	Johanna Moller, M.D.	Sr. Staff Fellow	MBDS, LMCN, NINDS
	Paul Durr	Biologist	MBDS, LMCN, NINDS
	Jeffrey Hammer	Biologist	MBDS, LMCN, NINDS
	Carl Lauter	Chemist	MBDS, LMCN, NINDS

COOPERATING UNITS (if any)

Department of Neurology, Johns Hopkins University, Baltimore, MD;
 Medical Neurology Branch, NINDS; Laboratory of Central Nervous System Studies, NINDS

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Myelin and Brain Development Section

INSTITUTE AND LOCATION

Park Building, Rm. 425, NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF YEARS:

1.3

PROFESSIONAL:

0.6

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was undertaken to elucidate biochemical and immunological aspects of myelin disorders associated with neuro-AIDS including myelin pallor, vacuolar myelopathy and multifocal demyelination in the CNS as well as demyelinating peripheral neuropathy. Postmortem CNS tissue from AIDS patients is being analyzed for quantitative and qualitative alterations of myelin proteins, including myelin-associated glycoprotein, myelin basic protein, proteolipid protein and 2',3'-cyclic nucleotide 3'-phosphodiesterase. The biochemical results are being correlated with histological and immunocytochemical observations made by our collaborators at Johns Hopkins University on adjacent sections of tissue. So far immunocytochemical staining of white matter of AIDS CNS has not suggested substantial loss of myelin proteins in areas where there is prominent myelin pallor, but a definitive conclusion on this must await the results of our quantitative biochemical studies currently in progress on AIDS cases with dementia in which myelin abnormalities are most common. It has been suggested that some of the myelin abnormalities in AIDS, and other neurological diseases caused by retroviruses, may be related to autoimmune phenomena. However, Western blotting experiments done this year have not revealed antibodies to proteins of CNS or PNS myelin in 10 sera from rabbits with HTLV-1 or 6 monkeys infected with SIV. In our previous studies on antibodies to gangliosides in the inflammatory peripheral neuropathies, one patient with high levels of serum antibodies to G_{D1b}-ganglioside was HIV-positive. Thus far, we have not detected anti-ganglioside antibodies in a limited number of additional AIDS patients with neuropathy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01N502848-01LMCN
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Disorders of CNS Myelin		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Johanna R. Moller, M.D.	Unit Head, Sr. Staff Fellow LMCN, NINDS
OTHERS:	Richard H. Quarles, Ph.D.	Laboratory Chief LMCN, NINDS
	Arun Chakrabarti, Ph.D.	Visiting Scientist LMCN, NINDS
	Masayuki Sasaki, M.D.	Special Volunteer LMCN, NINDS
	Paul Durr	Biologist LMCN, NINDS
	Carl Lauter	Chemist LMCN, NINDS
COOPERATING UNITS (if any) Developmental & Metabolic Neurology Branch, NINDS; Laboratory of Experimental Neuropathology, NINDS; School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin		
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology		
SECTION Demyelinating Disorders Unit, Section on Myelin and Brain Development		
INSTITUTE AND LOCATION Park Building, Rm. 425, NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: <div style="text-align: right;">1.8</div>	PROFESSIONAL: <div style="text-align: right;">1.2</div>	OTHER: <div style="text-align: right;">0.6</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Myelin basic protein (MBP)</u> and <u>proteolipid protein (PLP)</u> are major proteins of compact CNS myelin, whereas <u>myelin-associated glycoprotein (MAG)</u> and <u>2'3'-cyclic nucleotide 3'-phosphodiesterase (CNP)</u> are mainly localized in associated oligodendroglial membranes. These four myelin proteins are differently affected in various <u>dysmyelinating</u>, <u>demyelinating</u> and <u>remyelinating</u> circumstances, and information about changes in these proteins can increase our understanding of the specific molecular processes going on in each individual disease. In <u>multiple sclerosis (MS)</u>, there is a preferential loss of MAG at the edges of the plaques. Although there is weak cellular and humoral immunity to MAG in MS, it seems unlikely that this is the cause of the preferential loss of MAG. Much of the MAG remaining in MS tissue is in the form of <u>dMAG</u>, a proteolytic cleavage product apparently formed from its breakdown by a myelin-associated, <u>calcium-activated neutral protease</u>. The MAG loss in MS may be related to this protease. Experiments in which myelin purified from different species was incubated at 37°C in a neutral buffer demonstrated that the rate of dMAG formation was greatest in human myelin, rapid in myelin from other primates, and substantially slower in myelin from lower mammals such as rodents. This suggests that dMAG formation may be especially relevant to human diseases. Although there are active attempts at remyelination in MS, the newly formed myelin is also degraded. <u>Cuprizone</u>-intoxicated mice show a controlled demyelination and remyelination in the CNS. The basic biochemical processes in this model have been investigated, and factors that may affect remyelination such as the presence of anti-MAG antibodies will now be studied. In most <u>hypomyelinating mutant</u> animals, proteins of compact myelin (MBP and PLP) are decreased more than proteins in associated membranes (MAG and CNP), regardless of the primary cause of the hypomyelination. This is true in most demyelinating mutants, including a PLP gene defect (<u>shaking pup</u>), a cholesterol storage disorder (CSD mice), and a congenital virus infection (<u>Border disease</u> in sheep). This pattern of change in myelin proteins was also found in two human patients with <u>Niemann-Pick C</u> disease, and is probably due to a greater deficiency of compact myelin than of associated oligodendroglial membranes. At the moment, we are studying a new neurological rat mutant, the <u>TAIEP rat</u>, which is different in this respect. It expresses very low amounts of MAG in comparison to other myelin proteins, and the MAG is only detectable as the <u>immature large isoform</u>. We are also currently studying <u>brain biopsies</u> from two young girls with severe <u>hypomyelination</u> due to <u>unknown causes</u>. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01NS02864-01LMCN
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Reinnervation and Functional Recovery in Spinal Cord Injury		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: John W. Commissiong, Ph.D. OTHERS: Kotaro Shimoda, M.D. Takeo Takeshima, M.D., Ph.D. Helen Balling	Unit Head, Vis. Scientist Visiting Scientist Visiting Fellow Biologist	NTU, LMCN, NINDS LMG, NIMH NTU, LMCN, NINDS NTU, LMCN, NINDS
COOPERATING UNITS (if any) Medical Neurology Branch, NINDS		
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology		
SECTION Unit of Neural Transplantation		
INSTITUTE AND LOCATION Park Building, Room 415, NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.3	PROFESSIONAL: 1.3	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>When a complete cut of the <u>spinal cord</u> is made at the middle thoracic level in the neonatal rat at the 7th postnatal day (PN7) or earlier, the hindlimbs spontaneously recover the ability to make rhythmic steps, enabling the animal to <u>walk</u>, but with an ataxic gait. Recovery is minimal to absent when the <u>cordotomy</u> is done at PN14 or later. The results of H-reflex testing have demonstrated that Gp1a fibres make a strong functional contact with <u>alpha motoneurons</u> (α-MNs) in spinalized PN7 rats, but not in untreated PN14 rats. The α-MNs in the PN14 animals fire continually at 5-7 Hz, but do not respond, or respond very weakly to Gp1a stimulation. The receptive field for α-MNs of the triceps surae muscles also becomes greatly enlarged in spinalized PN7 rats. Treatment of the <u>spinalized</u>, PN14 rats with GM1 <u>ganglioside</u>, or injection of an E14 ventral mesencephalic (VM) cell suspension that contains 20% <u>tyrosine hydroxylase</u> positive (TH+) cells into the lumbosacral spinal cord, causes a degree of functional recovery in the spinalized PN14 rat that is statistically equivalent to the spontaneous recovery seen in the untreated PN7, spinalized rat. The lumbosacral <u>central pattern generator</u> (CPG) therefore displays a high degree of plasticity and is clearly capable of profound functional modification. There is a large body of neurophysiologic results detailing the modification of the lumbosacral CPG by <u>catecholamines</u>. The <u>transplantation</u> of fetal <u>dopaminergic neurons</u> into the lumbosacral spinal cord of the spinalized rat, has produced prolonged functional recovery in the spinalized PN14 rat, effectively duplicating the transient effects observed after the acute <i>in vivo</i> administration of catecholamines or their agonists. A dense reinnervation of the lumbosacral spinal cord with TH+ varicosities is observed after injection of the VM cell suspension into the spinal cord of the PN14 rat, although neuronal survival is poor. A similar phenomenon has also been observed <i>in vitro</i>, in which >95% of the plated cells die by DIV14. However, the TH+ cells that survive elaborate enormous dendritic arborizations. There is also a selective survival of TH+ cells in the culture after DIV14, when >60% of the surviving neurons are TH+. Our preliminary evidence suggests that a <u>neurotrophic factor</u> (NTF) is elaborated specifically by <u>astrocytes</u> of the ventral mesencephalon, and rescues the TH+ cells that survive after DIV12 from death, and promotes elaborate dendritic growth. We intend to isolate and molecularly characterize of the NTF that appears to be specific for rescuing dopaminergic neurons from death. We will use this NTF to attempt to enhance the survival of dopaminergic neurons <i>in vivo</i> after transplantation, and study the effects produced on <u>functional recovery</u>.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01NS01309-27LMCN
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biosynthesis and Function of Glycosphingolipids and Other Glycoconjugates		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Peter H. Fishman, Ph.D. OTHERS: Palmer Orlandi, Ph.D. Hema Patel	Chief, Membrane Biochemistry Section Research Associate Co-op Education Program	LMCN, NINDS MBS, LMCN, NINDS MBS, LMCN, NINDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology		
SECTION Membrane Biochemistry Section		
INSTITUTE AND LOCATION NINDS, NIH, Park Building, Room 411, Bethesda, MD. 20892		
TOTAL STAFF YEARS: 1.8	PROFESSIONAL: 1.3	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Ganglioside G_{M1} is the cell surface <u>receptor</u> for <u>cholera toxin</u> (CT). The pentameric B subunit of CT binds to G_{M1} whereas the A subunit of CT is involved in activation of <u>adenylyl cyclase</u>. The A subunit is reduced to the A₁ peptide which ADP-ribosylates the stimulatory <u>G protein</u> (G_s) of the cyclase. The orientation of CT when it binds, the pathway by which the A subunit enters cells and is reduced to A₁, and how the latter gains access to G_s have not yet been established. We have succeeded in clarifying some of these aspects using human intestinal Caco-2 cells, which behave in culture as differentiated enterocytes, the natural target for CT.</p> <p><u>Orientation of CT:</u> We were able to assemble active CT at the cell surface by sequentially exposing cells to the inactive B and A subunits. Based on the known structure of CT, we conclude that CT binds to cells with its A subunit facing away from the membrane. Using antibodies to A₁ and B, we were able to demonstrate that both subunits are internalized by cells.</p> <p><u>Intracellular Processing of CT:</u> To pursue the further processing of the internalized CT, we employed specific blockers. <u>Chloroquine</u> and <u>monensin</u>, which inhibit receptor-mediated <u>endocytosis</u> through coated pits, had no effect on CT action. By contrast, <u>brefeldin A</u>, which causes disassembly of the <u>Golgi apparatus</u>, was a potent blocker of CT action. Although brefeldin A did not prevent the internalization of CT, it did prevent its conversion to the A₁ peptide. As the latter is an essential step in the action of CT, brefeldin A may be a useful probe for delineating the intracellular processing of CT.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01NS02366-14LMCN
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Hormone-Responsive Adenylate Cyclase		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Peter H. Fishman, Ph.D. OTHERS: Michael Pak, Ph.D. Eric A. Gordon, Ph.D. Xiao-Ming Zhou, M.D., Ph.D. Patricia Curran Deborah Kauffman Ami Macurdy	Chief, Membrane Biochemistry Section Senior Staff Fellow Senior Staff Fellow Visiting Fellow Biologist Biologist Chemist	LMCN, NINDS MBS, LMCN, NINDS MBS, LMCN, NINDS MBS, LMCN, NINDS MBS, LMCN, NINDS MBS, LMCN, NINDS MBS, LMCN, NINDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular and Cellular Neurobiology, BNP		
SECTION Membrane Biochemistry Section		
INSTITUTE AND LOCATION NINDS, NIH, Park Building, Room 411, Bethesda, MD. 20892		
TOTAL STAFF YEARS: <div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px 10px; margin-right: 10px;">5.5</div> </div>	PROFESSIONAL: <div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px 10px; margin-right: 10px;">3.5</div> </div>	OTHER: <div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px 10px; margin-right: 10px;">2.0</div> </div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%; text-align: center;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Exposing cells to β-adrenergic agonists results in multiple effects on the <u>β-adrenergic receptor-coupled adenylyl cyclase</u>. The receptors rapidly become desensitized and less efficient at stimulating adenylyl cyclase; then the receptors become sequestered and inaccessible to hydrophilic ligands; and finally, the receptors are down-regulated as evidenced by a loss of antagonist binding. We previously found that the endogenous β_1-adrenergic receptors expressed by human neurotumor SK-N-MC cells are resistant to <u>desensitization</u> by the <u>β-adrenergic receptor kinase</u>. The latter has been shown to desensitize the human β_2-adrenergic receptor. We also observed that the β_1 receptors are resistant to <u>down-regulation</u> but undergo <u>sequestration</u>. In order to explore possible differences in the regulation of the two receptor subtypes, we transfected BHK and CHW hamster cells with an expression vector into which we inserted a cDNA encoding one or the other receptor subtypes, and isolated stable receptor-expressing transformants. In addition, we obtained transfected β_1- and β_2- CHO hamster cells and β_2-L mouse cells from other laboratories. The different cell lines were exposed to the agonist isoproterenol for different times and assayed for sequestration, down-regulation and desensitization. In all the transfected cell lines tested, β_2 receptors underwent sequestration faster and more extensively than β_1 receptors. Down-regulation was much more complicated and appeared to be affected by cell type. Although we have only been able to thoroughly assess three cell lines for desensitization, the results are encouraging. When cells were exposed to agonist for 30 min, the two cell lines expressing β_1 receptors exhibited no significant reduction in maximum stimulation of adenylyl cyclase by agonist. By contrast, the one β_2 receptor-expressing cell line tested so far underwent a 43% desensitization. Although these results are incomplete, they suggest differences in agonist-mediated regulation of human β_1- and β_2-adrenergic receptors, particularly sequestration and desensitization. These differences may relate to structural differences in the two receptors, especially their C-termini.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS02784-04LMCN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function Relationships in Cellular Signal Transduction Mechanisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert V. Rebois, Ph.D.	Head, Unit on Receptor Structure & Function	LMCN, NINDS
OTHERS:	Shigeru Okuya, M.D., Ph.D.	Visiting Associate	LMCN, NINDS
	V.J. Bhasker Reddy, Ph.D.	Visiting Fellow	LMCN, NINDS
	Nirmal S. Basi, Ph.D.	IRTA Fellow	LMCN, NINDS
	Dennis Warner, Ph.D.	IRTA Fellow	LMCN, NINDS
	M. Toyoshige, M.D., Ph.D.	Visiting Fellow	LMCN, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Membrane Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Park Building, Room 408, Bethesda, MD. 20892

TOTAL STAFF YEARS:

3.6

PROFESSIONAL:

3.6

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The stimulatory G protein (Gs) mediates the activation of hormone- and neurotransmitter-responsive adenylyl cyclase. Gs consists of an α subunit (Gs α) which has an intrinsic GTPase activity, and β and γ subunits that are tightly associated. Extensive research has produced a well-established model describing the molecular events that are mediated by Gs during hormone or neurotransmitter stimulation. Activation is initiated when the agonist-receptor complex promotes the exchange of GDP bound to Gs α for GTP. Nucleotide exchange in turn leads to dissociation of the Gs α from the $\beta\gamma$ -subunit complex. Subunit dissociation is believed to be pivotal since G $\beta\gamma$ can inhibit the activity of Gs α . Gs α activates adenylyl cyclase until GTP is hydrolyzed, whereupon G $\beta\gamma$ reassociates with Gs α and prevents further activation of adenylyl cyclase until another round of nucleotide exchange can trigger subunit dissociation. To investigate the role of subunit dissociation on Gs activation, the heterotrimeric protein was partially purified from bovine brain and rabbit liver. Complete and irreversible activation of Gs was achieved by incubating it with the nonhydrolyzable GTP analogue, GTP γ S, in the presence of different MgCl₂ concentrations. Activation was assessed by reconstitution of adenylyl cyclase activity in S49 cyc-membranes (which lack Gs α), and by measuring [³⁵S]GTP γ S binding to Gs. Subunit dissociation was determined by immunoprecipitating Gs α , and measuring the amount of β -subunit that coprecipitated. By these assays, it was determined that high concentrations of MgCl₂ caused subunit dissociation even in the absence of GTP γ S. When Gs was activated with low MgCl₂ concentrations, there was little or no subunit dissociation. It was also determined that the time course for Gs activation was more rapid than that of subunit dissociation. These data suggest that subunit dissociation may be unrelated to Gs activation, and indicate that the model for Gs activation may need revision.

ANNUAL REPORT

October 1, 1991 through September 30, 1992

Biometry and Field Studies Branch
Division of Intramural Research
Clinical Neurosciences Program
National Institute of Neurological Disorders and Stroke

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ANNUAL REPORT
October 1, 1991 through September 30, 1992

Biometry and Field Studies Branch
Division of Intramural Research
Clinical Neurosciences Program
National Institute of Neurological Disorders and Stroke

Jonas H. Ellenberg, Ph.D., Chief

The Biometry and Field Studies Branch (BFSB) supports a program in biostatistics to advance the mission of NINDS. The Branch participates in a wide range of intramural and extramural collaborative projects, including large- and small-scale observational studies, clinical trials and laboratory studies. These collaborative studies have been conducted both through direct staff projects and research and development contracts. In addition to collaborative work, the Branch has an important research component in statistical methodology. The Branch is composed of an Office of the Chief and three Sections. Dr. James Dambrosia is Deputy Chief of the Branch. In that capacity he assists the Branch Chief in policy decisions and assumes part of the administrative responsibilities of running the Branch.

The Mathematical Statistics Section (MSS), headed by Dr. James Dambrosia, is responsible for most of the collaborative statistical efforts with the other branches and laboratories in the Division of Intramural Research, the Extramural Research Divisions as well as neuroscience units outside of NINDS. The Branch has long recognized the need to focus resources specifically on the measurement of neurological outcome. To that end, a new Section has been created to replace the dormant Data Processing Section. The new Section, the Analytical Biometrics Section (ABS), will develop and implement a program for development and application of statistical methods to the measurement of biological processes including spatial and temporal phenomena in neurology and the neurosciences. Dr. Gregory Campbell, a senior statistician from DCRT, has joined BFSB as Chief of the new Section. Both the MSS and the ABS are responsible for research in statistical methodology and a significant degree of interaction between the two sections is expected.

The Collaborative Studies Section (CSS) has focused over the past ten years primarily on the development, implementation and analysis of two long term observational studies, the Stroke and the Traumatic Coma Data Banks. With the closeout of these studies and most of the primary analyses completed, the Section is exploring potential collaborative efforts as a statistical coordinating center for several new projects. These projects include studies of the etiology of Parkinson's disease in existing data bases, and a clinical trial of prophylaxis for second nonfebrile seizures in children. With the departure of the Section Chief, Dr. Mary Foulkes, to the Division of AIDS, NIAID, the Branch Chief is the Acting Chief of the Section.

Over the past three years, the Branch has aggressively recruited many recent Ph.D. statisticians for entry positions in the Staff Fellow Program. As a

result of these efforts and the recently approved Special Pay Rates for entry level Mathematical Statisticians, we have been able to recruit and retain several new young staff members. These include Dr. Robert Abel from Ohio State University, Dr. Paul Albert from Johns Hopkins University and Dr. Lisa McShane from Cornell University. With the departure of Dr. Foulkes and support staff members in the CSS, the Branch is operating at 13.95 full time equivalent positions, considerably below our current employment ceiling. We have as a goal, the recruitment of one additional staff fellow, one senior visiting statistician and a master's level biostatistician to adequately staff the CSS and ABS as they increase their activity to expected levels.

Several of our previous projects were long-term and labor-intensive, requiring a large amount of effort on the part of in-house staff for activities such as data entry, computer programming, systems analysis, data editing and day-to-day monitoring of protocol compliance. Data management support for collaborative studies is provided by two sources: in-house staff (a computer systems analyst located administratively in the CSS and a computer programmer located in the ABS); and to a large degree by an R&D contract, "Statistical and Collaborative Biomedical Research Data Management Support" (N01-NS-2-2320). This R&D contract, re-awarded to Information Management Services, Inc. on December 30, 1991, has a funding period of five years. The contract provides expertise in statistical programming, data entry, data monitoring, and systems analysis, in support of both collaborative projects and statistical methodologic research.

I. STATISTICAL COLLABORATION AND CONSULTATION

Our current program of collaborative research has developed primarily in response to requests for collaboration from intramural and extramural scientists at NINDS and from researchers outside of NIH. Typically, BFSB assumes responsibility for the statistical design, data management, statistical analysis, and interpretative aspects of the projects, with the subject matter specialists providing the project initiatives, subject matter expertise, and overall leadership. The Branch selects projects on the basis of scientific merit, a high probability of successful completion, and potential for scientific contributions consistent with the goals of the DIR.

In collaboration with the Division of Convulsive, Developmental and Neuromuscular Disorders (DCDND), and the Neuroepidemiology Branch (NEB), BFSB was the statistical coordinating center for the clinical trial of behavioral and cognitive side effects of phenobarbital used for the prevention of febrile seizure recurrence. This trial required extensive monitoring of patient accrual, extensive data quality control and several interim data analyses for the trial's Safety and Data Monitoring Committee. The primary results of this clinical trial have been published and the results of other analyses such as the effect of phenobarbital on sleep of children with febrile seizures, and the prediction of recurrence of febrile seizures, have been submitted for publication.

A second collaborative effort with the DCDND and the NEB is a population-based study of the prognostic value of the EEG for subsequent seizure activity in

children who experienced a febrile seizure. The cooperating medical center is the Pediatric Clinic in Skopje, Macedonia (Yugoslavia). The recruitment of new cases ended in December 1984, and follow-up (including repeat EEGs and neurologic and physical examinations) continued through FY 1991. The study includes 400 children with a normal or nonspecific abnormal EEG following a first febrile seizure, as well as about 300 children with a specific abnormal EEG following a seizure. The major outcomes of the study are recurrent febrile and afebrile seizures and their relationship to the initial EEG, subsequent EEG changes, and the influence of other medical and demographic factors. Univariate statistical analysis of the data for the baseline visit examined a large number of potential factors predictive of abnormal specific EEG classification. For example, the number of previous febrile seizures was associated with an increased rate of EEG abnormality: 18% in children with no previous seizures and 63% in those with four or more prior attacks. When these factors were considered jointly in a logistic regression model, the significant prognostic factors for abnormal specific EEG were: age at initial EEG; number of prior febrile seizures; focal febrile seizures; and motor activity abnormalities. Data editing for the follow-up visits is now complete and work is in progress on the assessment of the EEG as a predictor of recurrence and the changes of the EEG over time in children with febrile seizures.

The BFSB continues to collaborate with many Branches and Laboratories in the Division of Intramural Research (DIR). The feasibility of a randomized controlled trial of treatment with anticonvulsant medication following a first convulsion in children is being evaluated. This collaborative study involving BFSB, the NEB and clinical centers in Israel will address the issue of whether early treatment after a first seizure reduces the likelihood of developing chronic epilepsy. BFSB would collaborate as the statistical coordinating center for this project. BFSB is also collaborating on other clinical trials with the Medical Neurology Branch: one trial is evaluating the effect of felbamate in controlling seizures in adults with intractable partial epilepsy; the other is assessing its efficacy in children with Lennox-Gastaut syndrome.

Other collaborative studies in DIR include: development of optimal sampling procedures for estimation of the size of a population of neurons (Clinical Neuroscience Branch); mapping of the cerebral cortex using EMG amplitude and latency responses to electromagnetic stimuli to the scalp (Medical Neurology Branch); a clinical trial of high-dose prednisone in the treatment of post-polio muscular atrophy (Medical Neurology Branch); a case-control study of the potential association of serologically confirmed infection during pregnancy with morbidity in the child; a study of the effect of cholesterol-lowering agents on the course of Type-C Niemann-Pick disease (Developmental and Metabolic Neurology Branch); examination of the relationship between MRI lesion changes and clinical status in relapsing-remitting MS (Neuroimmunology Branch); examination of catecholamine, neuropeptide and amino acid levels in epilepsy patients at baseline and postictal periods (Medical Neurology Branch); survey of attitude and potential behavior of patients with von Recklinghausen's neurofibromatosis with respect to genetic screening (Neuroepidemiology Branch); assessment of time-to-motor response complication in L-dopa treated patients with Parkinson's disease (Experimental Therapeutics Branch); a prevalence study of neurologic diseases in the Navajo tribe

(Epilepsy Branch); measurement of hyperarousal in chronic insomnia patients (Medical Neurology Branch); a study of epilepsy progression to generalized tonic-clonic seizures (Medical Neurology Branch); clinical course and staging of Niemann-Pick disease (Developmental and Metabolic Neurology Branch); and determination of the effect of time from last seizure and seizure type on the dynamics of interictal metabolic change (Medical Neurology Branch).

Two clinical trials of glucocerebrosidase for treatment of Gaucher's disease are being done in collaboration with the Developmental and Metabolic Neurology Branch: one evaluates the effect of placental derived glucocerebrosidase (CeredaseTM) with recombinant β -glucocerebrosidase (rGCR) for bioequivalence; and the other compares the efficacy of two low-dose regimens of CeredaseTM, one with adjuvant vitamin D.

In collaboration with the Medical Neurology Branch, BFSB is providing computer database expertise in the development of a prototype database to conduct spatial searches of PET/MRI scans. This prototype will employ frontier spatial and object-oriented database technology for the first time in a medical application. Written in C++, the prototype database will employ the extensible database toolkit, Exodus, developed at the University of Wisconsin, and a spatial database C++ class library developed by Dr. Jack Orenstein, Object Design, Inc. Besides using novel computer technology, this project is unique in its emphasis on developing standard techniques for registering PET/MRI scans, and for transforming the registered images to a common stereotaxic coordinate system so that groups of patients' scans can be studied statistically. In order to identify local maxima associated with a specific task (e.g., moving the patient's right finger), an analysis of covariance procedure has been introduced. The prototype spatial database is being developed to permit comparison of local maxima different studies. When fully operational, spatial searches of a particular region of the brain will be able to be done by highlighting a region of a digitized brain atlas and searching for local maxima from all studies in a database.

Collaborative research with biomedical research units not in NINDS includes: a case-control repeated measurement study of "wearing out" syndrome in concert pianists; development of laboratory quality control procedures for the measurement of selenium; evaluation of the effects of weather and ambient light on mood in patients with seasonal affective disorders; area surveys for epidemiologic studies of neurologic disorders in Latin America, India and Italy; a study of the incidence of primary intracranial neoplasms in Israel; and a validation of consultations provided by U.S. drug information centers.

A major commitment has been made for collaboration with the Parkinson's Epidemiology Research Committee (PERC), a group comprised of individuals from the fields of movement disorders (neurology), biometry, epidemiology, occupational health, chemistry, toxicology and neuropathology. PERC is charged with both implementing and acting as a catalyst for the development of research protocols to identify industrial, agricultural and naturally occurring environmental chemicals and compounds that might play a role in Parkinson's disease (PD). PERC has evolved beyond its original "think tank" mission to become a working group, and the members have taken full advantage of the multidisciplinary nature of the committee. PERC has taken on the

feasibility evaluation or initiation of several specific projects, such as: (1) establishing a central registry to study PD clusters; (2) helping to establish a research mechanism for utilizing the patient database of the California Kaiser Permanente HMO systems to study the etiology and progression of PD; (3) using the resources of the Mayo Clinic for studies of etiology and progression of PD; (4) identifying chemicals in natural products that may cause PD; (5) determining if occupational exposure to MPTP-like chemicals increases the risk of PD; (6) examining available data bases to determine their utility in studying either the etiology or progression of PD; (7) summarizing what is known about the etiology of PD in a comprehensive, critical overview of the literature; and (8) assessing the feasibility of a prospective cohort study of the neurodegenerative disorders.

BFSB has taken the primary role for projects (6) (7) and (8). A comprehensive assessment of the Twins Registry of the Medical Follow-up Agency (MFA) of the Institute of Medicine (16,000 white male twin pairs, of service age during World War II), indicated that it is an unique resource for the study of gene-environment interactions, development, progression, and risk factors of neurodegenerative disorders. The twin registry provides an opportunity over the relative short term to address several research objectives, including: (i) assessment of the contribution of genetic factors to etiology; (ii) evaluation of the etiologic role of environmental, physiological, psychological and co-morbidity factors by comparison of differences in these factors between symptomatic and asymptomatic co-twins; (iii) elucidation of disease progression, by comparison of neuronal degeneration using in vivo measures under development in relation to clinical progression, in symptomatic and asymptomatic co-twins; and (iv) comparison of pathology of discordant twin pairs for insights into disease progression and etiology. The statistical design and analysis of a prospective study in this cohort provides a major challenge to take advantage of this nonrenewable resource in the most efficient and expeditious manner as is possible. A cooperative agreement, with BFSB as the statistical coordinating center, has been submitted by the California Parkinson's Foundation to NINDS for possible funding.

We have now compiled approximately 2000 papers related primarily to the etiology of PD, and the critical review and annotation for project (7) is almost complete. The published studies have not, in general, been definitive due to such problems as: difficulty of diagnosis; modest prevalence; and retrospective and environmental histories requiring long-term memory recall in PD patients or their surrogates. We are proceeding with the evaluation and annotation of the previous research, based on stated hypotheses, appropriateness of study design, and inference. Dr. Ellenberg will be senior editor of a book to be published by Marcel Dekker on the critical assessment of this knowledge base for etiology of PD. The book will also attempt to establish directions for promising future research.

Initiative (8) involves a large-scale long-term prospective study of a population of unaffected individuals, who will be assessed for risk factors and presymptomatic disease: these individuals will then be followed over time to accurately evaluate putative risk factors and their link to causality and to assess markers of presymptomatic disease. There are numerous research

questions that could be answered by a large-scale prospective, observational study of human neurodegenerative diseases, particularly those of aging, and only a few examples are given here: are there endogenous risk factors for human neurodegenerative diseases that can be identified in the genetic code?; are these risk factors amenable to mass screening?; are there environmental factors related to onset or time of onset of disease?; is it possible to identify individuals with presymptomatic disease, and if so, how far in advance of the onset of illness can this be done?; can the course of presymptomatic disease be accurately monitored, and if so, is it possible to alter the course of the disease?; will identification of genetic susceptibility factors lead to the identification of one or more causative agents?; will the identification of the disease process close to its inception enhance efforts to identify exogenous trigger factors and/or causative factors, and if so, will primary disease prevention become possible? BFSB in collaboration with PERC is currently involved in the design and assessment of the feasibility of this project.

Results from the Stroke Data Bank include: the time of day for onset for both intracerebral hemorrhage and subarachnoid hemorrhage patients with a history of hypertension is similar to the diurnal variation in blood pressure; demographic, medical history and clinical features, such as arm weakness, hypertension, diabetes, reduced consciousness at onset, and male gender, differentiate between severe atherosclerotic stenosis and cardioembolism, which could be useful in identifying eligible patients for hyperacute therapy; intraventricular extension and hematoma volume differentiate lobar from deep primary supratentorial intracerebral hemorrhage; diminished level of consciousness, visual field abnormalities, neglect aphasia, and other non-language cognitive dysfunctions were neurologic signs shown to be relevant to the diagnosis of cardiogenic embolism; and hemiparesis without sensory or cortical deficits was inversely associated with a cardiac source of embolism.

Results from the Traumatic Coma Data Bank include: patients within the Traumatic Coma Data Bank severely injured by civilian gunshot wounds were more likely to suffer a poor outcome if they had intracranial hypertension, midline shift on CT scan, compressed cisterns, subarachnoid blood, intraventricular hemorrhage, or hyper or mixed density lesions >15cc; neither the caliber of the gun nor the distance from the head significantly affected the risk of dying among those who survived long enough to be admitted for medical treatment; children with severe head injury, by contrast with adults, had higher mortality rates associated with diffuse brain swelling; age, length of coma, speed for both attending and motor movements, spatial integration and intact vocabulary were all significantly related to returning to work or school; a study of the relationship between performance on neuropsychological exams and functional status, intended to aid clinicians in making reliable assessments of outcome and appropriate recommendations for long-term care, showed that of the 19 neuropsychological measures recorded, Controlled Oral Word Association, Grooved Pegboard, Trailmaking Part B, and Rey-Osterrieth Complex Figure Delayed Recall were highly predictive of Glasgow Outcome Score.

II. METHODOLOGICAL RESEARCH IN STATISTICS

BFSB statisticians continue to develop new statistical methodology and derive innovative modifications of statistical techniques to meet the needs of the Institute for the design of experiments and field studies, analysis of data, and statistical modeling of biological processes and phenomena. Most of the statistical problems addressed arise from collaborative studies with the Division of Intramural Research and neuroscience units outside NINDS. In general, there are two objectives associated with these various statistical activities of BFSB. The primary objective is the development and improvement of statistical methodology to meet the needs of the Institute. The secondary objective is to make contributions to the development of statistical methodology which may be more generally useful in neurologic and other medical research.

A partial listing of areas in which BFSB staff is developing new statistical applications to neurologic problems includes: Markov models with an extended state space; identification of D-optimal designs for multilinear models; statistical analysis of shapes with spatial dependencies; growth functions for responses with contributions from two compartments; sampling strategies for rare neurologic disorders; methods of inference on frequency of events in follow-up data; analysis of response surfaces with spatially correlated errors; analysis of longitudinal data with missing observations; and statistical designs for two-state episodic diseases using follow-up data.

Theoretical statistical work has included: comparison of mixture model parameter estimates obtained by exact likelihood methods and EM methods; an empirical Bayes approach for examining multiple time series; derivation of consistent, efficient estimators for the bivariate Weibull distribution; Markov mixture models for time series count data; regression models for interval censored time-to-event data; analysis of clinical trial results with data missing in a nonrandom manner; optimal checking methods for laboratory quality control; use of eigenvalue decompositions of large multiway arrays for initial estimates for alternating least squares estimates; and methods of analyses for ordinal time series data.

III. BRANCH RECOGNITION AND OTHER PROFESSIONAL ACTIVITIES

Several of our staff have been active on important national and international review committees, and have participated in major meetings or received other peer recognition this fiscal year. Dr. Ellenberg serves on the Steering Committee of the Parkinson's Epidemiology Research Committee, and continues on the NIH, ad hoc Epidemiologist and Statistician Review Panel. Dr. Dambrosia is the Biometrics Society (Eastern and Western North American Region) representative to the AAAS Medical Science Section (N), is the Committee of Presidents of Statistical Societies' (COPSS) representative to AAAS, is the Chair of the Regional Advisory Board of the Eastern North American Region of the Biometrics Society, has been appointed General Methodology Chair for the 1993 Annual meetings of the American Statistical Association, serves on the Editorial Board of *Acta Neurologica Scandinavica* and was honored this year with the Public Health Service Special Recognition award for his significant

contributions to design and analysis of research studies in neurology. Dr. McShane presented an invited paper to the Biometric Society (ENAR) on "Accuracy of biochemical measurements for epidemiologic and nutrition studies," and to the American Statistical Association - American Society for Quality Control Technical Conference an invited paper on "Outgoing quality for continuous sampling plans." Dr. Anderson serves on the American Statistical Association's Committee on Committees and was an invited lecturer at scientific meetings in Portugal and Spain speaking on case-finding strategies for community-based surveys and methods for studying geographical clusters of neurologic disorders. Dr. Albert gave an invited lecture at the 1992 Eastern North American Region Biometrics Society meeting on "Statistical methods in the neurosciences: A Markov-model for ordinal repeated measures data in relapsing remitting disease." Dr. Foulkes is a member of the Monitoring Committee for the VA Cooperative Study of Carbamazepine versus Valproic Acid for Treatment of Partial Seizures, was elected Secretary of the Eastern North American Region of the Biometric Society, was appointed Program Chair for the 1993 Annual meetings of the American Statistical Association, was awarded the 1991 Washington Statistical Association President's Award and was awarded the NIH Director's Award for her leadership of the NINDS Stroke and Traumatic Coma Data Banks.

In summary, BFSB is involved in a strong program of collaborative research. Our collaboration extends throughout the Institute on projects with both intramural and extramural scientists, and also involves collaboration with scientists outside of NINDS. The scope of our research activity ranges from small, one-on-one collaboration with intramural scientists, to the conduct of large-scale, multicenter clinical studies. BFSB also makes an important and continuing contribution to statistical methodology applicable to neurologic research.

CONTRACT NARRATIVE
Biometry and Field Studies Branch, CNP, DIR, NINDS
Fiscal Year 1992

Information Management Services, Inc., Rockville, Maryland
(N01-NS-2-2320)

Title: Statistical and Collaborative Biomedical Research
Data Management Support

Date Contract Initiated: December 30, 1991

Contractor's Project Director: William Lake, Jr.

Current Annual Level FY 92: \$61,204

Objectives: To provide statistical programming and data management support for both collaborative research projects and the development of statistical methodology.

Major Findings: This Contract, a 5-year continuation of Contract N01-NS-9-2325, provides statistical programming and data management support for data entry, editing, quality control and report generation for all BFSB collaborative projects. Software for new statistical methods as well as all data management support is developed, tested and implemented by the Contractor on the NIH computer system.

Significance to the NINDS Program and Biomedical Research: The statistical staff of BFSB engages in collaborative biomedical research and conducts statistical research evolving generally from problems encountered in these collaborative studies. The Contract provides timely and efficient systems development, data management (including data entry), data processing and programming for both ongoing and future collaborative studies. Statistical programming, an essential element of biostatistical research, is provided under the direction of the Branch. New statistical methods developed by BFSB are coded, evaluated and then made compatible with existing interactive statistical software packages by the Contractor.

Proposed Course of the Project: The Contract began on December 30, 1991 and continues through December 29, 1996.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02652-08 BFSB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Statistical Collaboration and Consultation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	James M. Dambrosia, Ph.D. Chief, Mathematical Statistics Section Chief	BFSB, DIR, NINDS BFSB, DIR, NINDS
Others:	Jonas H. Ellenberg, Ph.D. Mathematical Statistician Robert B. Abel, Ph.D. Mathematical Statistician Paul S. Albert, Ph.D. Mathematical Statistician Dallas Anderson, Ph.D. Mathematical Statistician Sherrie E. Emoto, Ph.D. Mathematical Statistician Mary A. Foulkes, Ph.D. Chief, Collaborative Studies Section Lisa McShane, Ph.D. Mathematical Statistician	BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS
COOPERATING UNITS (if any) Bombay Hospital, India (Dr. N. Bharucha); Peking Union Medical College, PRC (Dr. Z. Zhang); MIMH (Dr. Norman Rosenthal); Univ. of Chile, Santiago, Chile (Dr. V. Diaz); Foundation of Research in Neuroepidemiology Junin, Argentina (Dr. M. Melcon); Harvard Univ. Boston, MA (Dr. Q. Register); Istituto delle Malattie Nervose e Mentali, Rome, Italy (Dr. A. Pepe)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Office of the Chief, Mathematical Statistics Section, Collaborative Studies Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.0	5.0	2.0
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project encompasses a wide scope of statistical collaboration and consultation with Laboratories and Branches within the Division of Intramural Research (DIR), and with other neuroscience units outside NIH. Particular consideration is given to <u>statistical planning and design of experiments, statistical analysis of data, and statistical inference</u> . Examples of current studies include: clinical studies of cholesterol-lowering agents in Neimann-Pick disease, clinical course and outcome of patients with Neimann-Pick disease, clinical studies of Cerease TM in Gaucher's disease (Developmental and Metabolic Neurology Branch); clinical trials of felbamate for the treatment of intractable complex partial seizures, measurement of the effect of time from last seizure and seizure type on metabolic change as measured by PET, clinical trial of prednisone for the treatment of post-polio muscle atrophy, statistical analysis of shape and spatial relationships of maps of the cerebral cortex based on EMG responses to electromagnet stimulation of the scalp, evaluation of IV/IG efficacy in dermatomyositis and polymyositis, study of epilepsy progression to general tonic-clonic seizures, three clinical trials of IV/IG in neuromuscular disorders (Medical Neurology Branch); development of time series models for the effect of weather and light on mood in patients with seasonal affective disorder (NIMH); optimal sampling procedures to estimate the size of a population of neuron cells (Clinical Neuroscience Branch); examination of the relationship between MRI change and clinical status in relapsing-remitting MS, clinical trial of the effect of cyclosporine on lesion development in relapsing-remitting MS, modeling lesion recurrence in relapsing-remitting MS, evaluation of the effects of TGF- β and anti-TGF- β on chronic relapsing EAE (Neuroimmunology Branch); statistical modeling of time-to-motor response complication in L-dopa treated patients with Parkinson's disease (Experimental Therapeutics Branch); prevalence of neurologic diseases in the Navajo tribe (Epilepsy Branch); incidence study of primary intracranial neoplasms in Israel; validation study of consultations provided by U.S. drug information centers; case-control study of hemorrhagic stroke and alcoholism in Santiago, Chile; and a prevalence survey of major neurologic disorders in Junin, Argentina.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02490-12 BFSB									
PERIOD COVERED October 1, 1991 through September 30, 1992											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Research in Statistics											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 35%; vertical-align: top;"> PI: James M. Dambrosia, Ph.D. Co-PI: Jonas H. Ellenberg, Ph.D. Others: Robert B. Abel, Ph.D. Paul S. Albert, Ph.D. Dallas W. Anderson, Ph.D. Sherrie E. Emoto, Ph.D. Mary A. Foulkes, Ph.D. Lisa M. McShane, Ph.D. </td> <td style="width: 65%; vertical-align: top;"> Chief, Mathematical Statistics Section Chief Mathematical Statistician Mathematical Statistician Mathematical Statistician Mathematical Statistician Chief, Collaborative Studies Section Mathematical Statistician BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS </td> </tr> </table>			PI: James M. Dambrosia, Ph.D. Co-PI: Jonas H. Ellenberg, Ph.D. Others: Robert B. Abel, Ph.D. Paul S. Albert, Ph.D. Dallas W. Anderson, Ph.D. Sherrie E. Emoto, Ph.D. Mary A. Foulkes, Ph.D. Lisa M. McShane, Ph.D.	Chief, Mathematical Statistics Section Chief Mathematical Statistician Mathematical Statistician Mathematical Statistician Mathematical Statistician Chief, Collaborative Studies Section Mathematical Statistician BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS							
PI: James M. Dambrosia, Ph.D. Co-PI: Jonas H. Ellenberg, Ph.D. Others: Robert B. Abel, Ph.D. Paul S. Albert, Ph.D. Dallas W. Anderson, Ph.D. Sherrie E. Emoto, Ph.D. Mary A. Foulkes, Ph.D. Lisa M. McShane, Ph.D.	Chief, Mathematical Statistics Section Chief Mathematical Statistician Mathematical Statistician Mathematical Statistician Mathematical Statistician Chief, Collaborative Studies Section Mathematical Statistician BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS										
COOPERATING UNITS (if any)											
LAB/BRANCH Biometry and Field Studies Branch											
SECTION Mathematical Statistics Section											
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892											
TOTAL STAFF YEARS: 4.0	PROFESSIONAL: 3.0	OTHER: 1.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews		
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither									
<input type="checkbox"/> (a1) Minors											
<input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project addresses <u>statistical problems</u> generated from collaboration with scientists in other program areas and general statistical problems of current interest. This project is a continuing activity of the Section on Mathematical Statistics and other members of the Branch. Papers have been submitted, are in review or were published in FY 1992 on the following statistical subjects: eigenvalue decompositions of data modeled by multiway arrays; optimal experimental design for data modeled by three-way arrays; exact likelihood estimation of parameters in a Markov mixture model; computation of determinants of certain large information matrices; Bayesian methods for logistic regression on ill-behaved data; validation methods for screening instruments in surveys of low prevalence disease; national prevalence estimates of disease obtained by adjustment and incorporation of estimates from independent community-based surveys; empirical bayes procedure for examining the relationships among multiple time series; and influence of missing data in randomized clinical trials. Other work in progress includes: methods to improve coverage in surveys; estimation of time-to-event data with interval censoring; site selection for epidemiologic surveys; adjustments for covariates in the analysis of categorical data; two-state models for analyzing time series count data; analysis of response surface data with spatial and temporal components; sampling strategies for count data with multiple types of clustering; statistical models and analysis methods for time series of ordinal data; modeling of response surfaces with spatially correlated errors; application of splines to estimate model parameters of multiple correlated response surfaces; modeling effect changes of covariates in the presence of spatial correlation and combining information from negatively correlated nonlinear regressions.											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02483-12 BFSB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Predictive Value of the EEG in Febrile Seizures		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Sherrie E. Emoto, Ph.D.	Mathematical Statistician BFSB, DIR, NINDS
Others:	Jonas H. Ellenberg	Chief BFSB, DIR, NINDS
	Deborah G. Hirtz, M.D.	Health Science Admin. DNB, DCDN, NINDS,
	Karin B. Nelson, M.D.	Medical Officer NEB, DIR, NINDS
	Jack Panossian	Programmer BFSB, DIR, NINDS
	Dolores Jones	Computer Clerk BFSB, DIR, NINDS
COOPERATING UNITS (if any) Developmental Neurology Branch, DCDN, NINDS; Neuroepidemiology Branch, DIR, NINDS; Nikola Sofijanov, M.D., Pediatric Clinic, University of Skopje, Macedonia (Yugoslavia)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	0.6	PROFESSIONAL: 0.2 OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>This population-based study will evaluate the significance of the <u>EEG</u> as a predictor for recurrence of seizures in those children who have had a simple febrile convulsion. Outcomes reported are <u>febrile seizure recurrence</u> and <u>afebrile seizure occurrence</u>. The evolution of the EEG pattern will be described, and patterns will be correlated with the clinical outcome. The clinical study was carried out in Skopje, Macedonia (Yugoslavia), at the Pediatric Clinic of the University of Skopje.</p> <p>The study began in FY 1982. Patient accrual was completed in December, 1984, by which time approximately 400 patients with a febrile seizure, no prior complex or multiple seizures and with a normal or nonspecific abnormal EEG, were registered into the study and began the study protocol and follow-up. An additional 300 patients with a specific abnormal EEG were entered for baseline information and follow-up. Additional efforts by the clinical center were needed to collect data from those patients lacking a return visit and those who did not have long term follow-up. Final follow-up visits were completed in FY 1991. Data editing and file creation are complete. Statistical analysis of baseline EEG and its association with characteristics of the child and family and the clinical characteristics of the seizure has been published. Analysis is currently being conducted to examine: the effectiveness of the initial EEG in predicting recurrent febrile seizures; the evolution of EEGs in children with febrile seizures; and the value of changes in EEGs in predicting febrile seizure recurrence. In addition, information on comorbidity is being extracted from the original forms for future assessment.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02598-10 BFSB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Stroke Data Bank		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Mary A. Foulkes, Ph.D.	Chief, Collaborative Studies Section BFSB, DIR, NINDS
Others:	James M. Dambrosia, Ph.D.	Chief, Mathematical Statistics Section BFSB, DIR, NINDS
	Jonas H. Ellenberg, Ph.D.	Chief BFSB, DIR, NINDS
	Jack Panossian	Programmer BFSB, DIR, NINDS
	Alan Polis	Comp. Syst. Analyst BFSB, DIR, NINDS
COOPERATING UNITS (if any) Departments of Neurology: Boston University Medical Center, Michael Reese Hospital, Neurological Institute - Columbia University, and University of Maryland		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Collaborative Studies Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	0.8	PROFESSIONAL: 0.4 OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The <u>Stroke Data Bank</u> is a prospective observational study which collected data on hospitalized newly diagnosed <u>stroke patients</u> at four clinical centers. The collaborating clinical centers were responsible for the collection of acute care and longitudinal follow-up information on 1,805 patients using common definitions and procedures, under contracts NO1-NS-2-2302, NO1-NS-2-2398-9, NO1-NS-2-2384. The objective for the project was to provide a comprehensive body of data for clinical research on the factors influencing <u>survival</u>, <u>morbidity</u> and <u>quality of life</u> following <u>onset of a stroke</u>. The BFSB served as the statistical coordinating center for the project. An analysis of the time of day of stroke onset suggested that the time of onset for both intracerebral hemorrhage and subarachnoid hemorrhage patients with a history of hypertension is similar to the diurnal variation in blood pressure. Demographic, medical history and clinical features, such as arm weakness, hypertension, diabetes, reduced consciousness at onset, and male gender, have been shown to differentiate between severe atherosclerotic stenosis and cardioembolism, which could be useful in identifying eligible patients for hyperacute therapy. Similarly, the factors which differentiate lobar from deep primary supratentorial intracerebral hemorrhage were intraventricular extension and hematoma volume. The neurologic signs shown to be relevant to the diagnosis of cardiogenic embolism were diminished level of consciousness, visual field abnormalities, neglect aphasia, and other non-language cognitive dysfunctions. Hemiparesis without sensory or cortical deficits was inversely associated with a cardiac source of embolism. Continuing analysis of this data set will be reported under Intramural Project: Statistical Coordinating Center for Collaborative Clinical Studies (ZO1 NS 02810-03). The data set is available to the public through the National Technical Information Service (NTIS Order Number PB92-500313). This project is completed. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02516-11 BFSB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Traumatic Coma Data Bank		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Mary A. Foulkes, Ph.D. Others: Jonas H. Ellenberg, Ph.D. Jack Panossian Alan Polis	Chief, Collaborative Studies Section Chief Programmer Comp. Syst. Analyst	BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS BFSB, DIR, NINDS
COOPERATING UNITS (if any) Department of Neurosurgery: Medical College of Virginia, University of California - San Diego, University of Texas - Galveston, University of Virginia		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Collaborative Studies Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.4	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="display: flex; flex-direction: column; align-items: flex-start;"> <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> <div style="display: flex; align-items: center;"> <input type="checkbox"/> (b) Human tissues </div> <div style="display: flex; align-items: center;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The <u>Traumatic Coma Data Bank</u> is a prospective observational study which collected data on <u>severely head-injured</u> patients at four clinical centers. The collaborating centers were responsible for the collection of acute care and longitudinal follow-up information on 1,030 patients using common definitions and procedures, under contracts NO1-NS-3-2339-42. The general objective for the project was to provide a comprehensive body of data for clinical research on the factors influencing <u>survival</u>, <u>morbidity</u> and <u>quality of life</u> following a severe head injury. The BFSB was the statistical coordinating center for the project. Accrual of 1,030 patients was completed in September 1987, and patient follow-up was completed in January 1988. Children with severe head injury, by contrast with adults, had higher mortality rates associated with diffuse brain swelling. Outcome as a function of employment status or return to school was evaluated in severely head injured patients. Age, length of coma, speed for both attending and motor movements, spatial integration and intact vocabulary were all significantly related to returning to work or school. A study of the relationship between performance on neuropsychological exams and functional status, intended to aid clinicians in making reliable assessments of outcome and appropriate recommendations for long-term care, showed that of the 19 neuropsychological measures recorded, Controlled Oral Word Association, Grooved Pegboard, Trailmaking Part B, and Rey-Osterrieth Complex Figure Delayed Recall were highly predictive of Glasgow Outcome Score. Continuing analysis of this data set will be reported under Intramural Project: Statistical Coordinating Center for Collaborative Clinical Studies (ZO1 NS 02810-03). The data set is available to the public through the National Technical Information Service (NTIS Order Number PB91-509893). This project is completed. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02810-03 BFSB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Statistical Coordinating Center for Collaborative Clinical Studies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Jonas H. Ellenberg, Ph.D.	Acting Chief, Collaborative Studies Branch BFSB, DIR, NINDS
Others:	Karin B. Nelson	Medical Officer NEB, DIR, NINDS
	Jack panossian	Programmer BFSB, DIR, NINDS
	Alan Polis	Comp. Syst. Analyst BFSB, DIR, NINDS
COOPERATING UNITS (if any) Judith Manelis, M.D., Neurologist, Western Galilee Regional Hospital, Nahariya, Israel; Caroline Tanner, M.D., Neurologist, California Parkinson's Foundation; Sheldon Wolf, Neurologist, Kaiser-Permanente, Southern California		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Collaborative Studies Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	1.2	PROFESSIONAL: 0.8 OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project encompasses all statistical coordinating center responsibilities for <u>collaborative clinical studies</u> undertaken by this Section and the Office of the Chief. Current collaborative clinical studies being planned include a double-blind trial designed to examine the influence of <u>anticonvulsant therapy</u> on the natural history of <u>seizure disorders</u> in young patients. Whether treatment after the first seizure can alter likelihood of development of <u>chronic epilepsy</u> is a question often raised with no definitive answer. A number of observational studies establish that many persons who experience a first generalized convulsion do not experience a recurrence during two to five year periods of follow-up. Since prescription of anticonvulsant medication involves risk of side effects as well as expense, it might be desirable to withhold a commitment to chronic therapy until a second or subsequent seizure takes place. It is anticipated that the local clinics in Israel will fund the clinical aspects of the trial and design, establishment and maintenance of the master data file, monitoring and analysis will be the function of BFSB as the statistical coordinating center. </p> <p> A second collaborative study involves the study of the <u>etiology</u> of <u>Parkinson's disease (PD)</u> using the <u>twin pair registry</u> of the National Academy of Sciences/National Research Council. The prevalent cases of PD in the more than 6,000 twin pairs in which both members are alive, will be identified. This observational study will include: environmental, medical and family histories of both affected and unaffected members of the twin pairs; DNA banking; and measurement of progression of disease over time. This project will investigate genetic and environmental contributions and their interactions to the etiology of PD. A Cooperative Agreement has been submitted for funding of the clinical aspects of this study. BFSB will act as the statistical coordinating center. </p> <p> BFSB will collaborate on the development of the record linkage system of the Kaiser Permanente HMO (with several million members) so that the HMO may be used in the future as a research resource for testing of hypotheses on the etiology and progression of PD. </p>		
15 - BFSB/DIR		

ANNUAL REPORT

October 1, 1991 through September 30, 1992
Developmental and Metabolic Neurology Branch
Clinical Neurosciences Program, DIR
National Institute of Neurological Disorders and Stroke

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Synthesis of Inhibitors of N-Myristoyltransferase Z01 NS02816 03 DMN	20
Investigation of the Etiology Mucopolysaccharidoses IV Z01 NS 02843-01 DMN	21
Investigation of the Etiology of Batten's Disease Z01 NS 02844-01 DMN	22
Investigation of Enzyme Replacement Therapy in an Analogue of Human GM1-Gangliosidosis Z01 NS 02845-01 DMN	23

ANNUAL REPORT

OCTOBER 1, 1991 THROUGH SEPTEMBER 30, 1992
DEVELOPMENTAL AND METABOLIC NEUROLOGY BRANCH, DIR
NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

ROSCOE O. BRADY, M.D., CHIEF

The principal activities of the Branch include the following areas of investigation:

1. Basic studies of lipid and mucopolysaccharide synthesis and catabolism.
2. Identification of enzymatic abnormalities in hereditary metabolic disorders.
3. Investigations of the molecular basis of metabolic storage disorders.
4. Development of transgenic homologs of human disorders.
5. Pathogenic and therapeutic investigations in animal models of human disorders of metabolism.
6. Clinical investigations of lysosomal storage disorders and neurogenetic diseases.
7. Development of therapy for patients with heritable diseases.
8. Development of gene replacement technology.
9. Exploration of strategies for the treatment of AIDS and neoplastic diseases.

I. BASIC INVESTIGATIONS IN HEREDITARY METABOLIC DISORDERS

A. Gaucher's Disease

Fundamental studies have been carried out to augment the delivery of macrophage-targeted glucocerebrosidase to glucocerebroside-storing cells in patients with Gaucher's disease. The pharmacologic up-regulation of mannose lectins that are involved in this process has been documented, and the most effective agent is being examined in an enzyme replacement trial in patients with Type 1 Gaucher's disease.

B. Fabry's Disease

Human ceramidetrihexosidase (alpha-galactosidase A) has been produced by recombinant DNA technology. The production of this enzyme will be scaled-up in order to examine its clinical effectiveness in patients with Fabry's disease.

II. INVESTIGATIONS OF THE MOLECULAR BASIS OF METABOLIC STORAGE DISORDERS

A. Type C Niemann-Pick Disease

We have made excellent progress in our efforts to identify the molecular defect in Type C Niemann-Pick disease. We have strong evidence for the chromosomal localization of the gene that is mutated in this condition. We shall clone and sequence the normal gene and identify the gene product. We shall determine the mutation(s) that have occurred in this gene in patients with Type C Niemann-Pick disease.

III. PATHOGENIC AND THERAPEUTIC INVESTIGATIONS IN ANIMAL MODELS

A. Enzyme Replacement Trials in Feline and Canine Analogs of Human Generalized (GM1) Gangliosidoses

In a collaborative investigation with the Surgical Neurology Branch, CNP, DIR, NINDS, we are examining the effect of intracerebral injections of beta-galactosidase into animals with generalized (GM1) gangliosidosis. Investigators in the Surgical Neurology Branch have developed a technique for the delivery of materials to the brain that by-passes the blood-brain barrier. We are collaborating with these neurosurgeons to determine the effect of beta-galactosidase on the level of ganglioside GM1 that has accumulated in the brains of cats and in dogs with generalized (GM1) gangliosidosis that are deficient in this enzyme. If the level of accumulating ganglioside is reduced significantly by this treatment, we shall explore this approach for the therapy of patients with a number of metabolic disorders that involve the central nervous system.

IV. DEVELOPMENT OF TRANSGENIC ANIMAL MODELS OF HUMAN DISORDERS

A. Transgenic Mice Containing Mutated Growth Factor Genes

We have introduced a mutated non-coding gene (gene knock-out) for transforming growth factor-beta (TGF-beta) into the germ line of C57 mice. Chimeric mice carrying this mutation have been mated, and homozygous mice with a runted phenotype and limited survival have been produced that lack the TGF-beta 1. We are performing detailed pathological examinations to determine the role of TGF-beta 1 in growth and development.

V. CLINICAL INVESTIGATIONS OF METABOLIC DISORDERS AND NEUROGENETIC DISEASES

A. Type C Niemann-Pick disease

We have developed a highly informative neurologic staging system to evaluate the status and rate of disease progression in patients with Type C Niemann-Pick disease. A number of important findings that have prognostic significance have been discovered. We will use this information to evaluate the effects of therapeutic strategies that are developed to treat patients with this disorder.

B. Identification of a New Neurologic Disorder

We have documented a novel neurologic syndrome in young females that is characterized by mild ataxia coupled with severe alterations of magnetic resonance spectroscopy of the white matter of the brain. After a period of several years, the patients become nonambulatory and exhibit other characteristics of leukodystrophy including dysarthria and tremor. We are examining the biochemical and molecular biological alterations that occur in patients with this condition.

VI. THERAPY FOR HEREDITARY METABOLIC DISORDERS

A. Gaucher's Disease

The extraordinary effectiveness of macrophage-targeted human placental glucocerebrosidase for patients with Type 1 Gaucher's disease was established in a clinical efficacy trial carried out by the Developmental and Metabolic Neurology Branch. The salutary effects of enzyme replacement have been completely substantiated in numerous independent investigations. More than 600 patients with Gaucher's disease are now benefiting from this enzyme replacement therapy. We are currently determining the minimum amount of enzyme that is necessary to obtain a clinical benefit. We have recently shown that the quantity of enzyme that is required to maintain patients in good health is far less than the amount that is initially necessary to reverse the pathologic changes that occur in this disorder. We are also investigating the minimum frequency of enzyme administration that is required for clinical benefit and for maintaining the health of patients with Gaucher's disease. We are also examining the effect of enzyme replacement therapy in patients with Type 3 Gaucher's disease, the chronic neuronopathic form of this disorder. In addition, we are also investigating the clinical effectiveness of recombinantly produced macrophage-targeted glucocerebrosidase.

B. Type C Niemann-Pick Disease

A Phase 1 clinical investigation has been carried out to examine the feasibility of therapeutic agents to reduce circulating and tissue cholesterol levels in patients with Type C Niemann-Pick disease. The safety of the pharmacologic agents used in this initial study has been determined, and this information will be incorporated into a clinical efficacy trial for the treatment of patients with Type C Niemann-Pick disease.

VII. DEVELOPMENT OF GENE REPLACEMENT TECHNOLOGY

A. Gaucher's Disease

The gene for human glucocerebrosidase has been successfully transferred into mouse and human hematopoietic stem cells using a high-titer, helper-free recombinant retrovirus that we have constructed. Cells obtained from the bone marrow of patients with Gaucher's disease have been transfected and restoration of glucocerebrosidase activity equivalent to that in normal individuals has been achieved. The gene has also been efficiently transferred into murine hematopoietic stem cells. The vector genome is detected in all cells of hematopoietic lineage, and human glucocerebrosidase RNA is produced in all tissues of this lineage. Macrophages of long-term reconstituted mice produce human glucocerebrosidase in amounts equal to normal mouse endogenous levels. These findings provide strong encouragement for undertaking gene replacement therapy in patients with Gaucher's disease.

VIII. THERAPEUTIC APPROACH TO AIDS AND NEOPLASTIC DISEASES

We have synthesized a novel class of inhibitors of the enzyme N-myristoyltransferase. This enzyme catalyzes the covalent addition of myristic acid to the N-terminal glycine of the gag and nef polypeptides of HIV. Blocking n-myristoylation of the gag polypeptide prevents the formation of new virus particles. The compounds that we have produced have been examined in N-myristoyltransferase assay systems. Those that inhibited the activity of the enzyme are being tested for anti-HIV activity. One of these compounds has been found to inhibit the growth of certain types of neoplastic cells in culture. We are now examining the antitumor activity of this substance in appropriate *in vivo* systems.

CONTRACT NARRATIVE

DEVELOPMENTAL AND METABOLIC NEUROLOGY BRANCH
DIVISION OF INTRAMURAL RESEARCH, NINDS
OCTOBER 1, 1991 THROUGH SEPTEMBER 30, 1992

Contractor: GENZYME CORPORATION, BOSTON, MA. (N01-NS-9-2365)

Title: Preparation of Glucocerebrosidase from Human Placental Tissue

Contractor's Project Director: F. Scott Furbish

Current Annual Level of Support: \$300,000

Objectives: To isolate human placental glucocerebrosidase in sufficient purity and quantity for use in enzyme replacement trials in patients with Gaucher's disease.

Major Findings: A procedure has been developed for the large-scale purification of human placental glucocerebrosidase targeted to cells in which glucocerebrosidase accumulated in patients with Gaucher's disease. The intravenous infusion of this enzyme has caused marked clinical improvement in patients with Gaucher's disease.

Significance to Biomedical Research and to the Program of the Institute:

A principal mission of the Institute is to develop effective therapy to treat human diseases. We have achieved this goal in patients with Type 1 Gaucher's disease, the most prevalent human metabolic storage disorder.

Proposed Course of the Contract: We shall determine the minimal amount of exogenous glucocerebrosidase required to stabilize patients in whom the beneficial responses indicated above have occurred. We shall also determine the minimal amount of enzyme required to bring about these salutary changes. We shall also determine the minimum frequency of enzyme infusions required to maintain patients in good health. In addition, we are examining enzyme replacement therapy in patients with Type 3 Gaucher's disease, the chronic neuropathic form of this disorder.

CONTRACT NARRATIVE

DEVELOPMENTAL AND METABOLIC NEUROLOGY BRANCH DIVISION OF INTRAMURAL RESEARCH, NINDS OCTOBER 1, 1991 THROUGH SEPTEMBER 30, 1992

Contractor: LIPITEK, INC. (NO1-NS-0-2386)

Title: Production of Radiolabeled Sphingolipids

Contractor's Project Director: Alexander L. Weis, Ph.D.

Current Annual Level of Support: \$85,200

Objectives: To prepare glucocerebroside, sphingomyelin, and ceramidetrihexoside labeled with ^{14}C in critical portions of the molecule for diagnostic tests for Gaucher's Niemann-Pick and Fabry's diseases.

Major Findings: Synthetic chemical procedures have been developed to incorporate radioactive ^{14}C in specific portions of sphingolipid molecules. These compounds are used to diagnose patients with the sphingolipid storage disorders listed above; identify heterozygous carriers of these conditions; diagnose these disorders prenatally; and monitor enzyme isolation procedures for glucocerebrosidase, sphingomyelinase, and ceramidetrihexosidase.

Significance to Biomedical Research and to the Program of the Institute:

The ability to diagnose patients, identify heterozygotes, and to monitor pregnancies at risk for sphingolipid storage disorders represents major contributions to the controlling of the incidence of these diseases. These procedures are widely used at the present time.

Proposed Course of the Contract: The contract has been awarded. Contractor's performance will be monitored for compliance under a fixed-price agreement concerning the requisite deliverables.

CONTRACT NARRATIVE

DEVELOPMENTAL AND METABOLIC NEUROLOGY BRANCH DIVISION OF INTRAMURAL RESEARCH, NINDS OCTOBER 1, 1991 THROUGH SEPTEMBER 30, 1992

Contractor: GENZYME CORPORATION, BOSTON, MA. (N01-NS-9-2358)

Title: Preparation of Ceramidetrihexosidase by Recombinant DNA Technology

Contractor's Project Director: F. Scott Furbish

Current Annual Level of Support: \$100,000

Objectives: To isolate human ceramidetrihexosidase in sufficient purity and quantity for use in enzyme replacement trials in patients with Fabry's disease.

Major Findings: A procedure is being developed for the large-scale production of human ceramidetrihexosidase by recombinant means in sufficient purity and specific catalytic activity so that it can be safely administered to patients with Fabry's disease. Its carbohydrate portion will be analyzed. Methods to target the enzyme to cells and tissues where ceramidetrihexoside is stored are being developed.

Significance to Biomedical Research and to the Program of the Institute:

A principal mission of the Institute is to develop effective therapy for human diseases. If beneficial clinical results can be obtained, an extraordinary milestone will have been accomplished regarding this type of a human genetic disease.

Proposed Course of the Contract: We have made significant progress in our effort to increase the delivery of this enzyme to specific cells in which ceramidetrihexoside accumulates. We shall resume enzyme replacement trials in patients with Fabry's disease when the recombinant enzyme is available. We shall examine the effectiveness of the enzyme with regard to clearance of accumulated ceramidetrihexoside in the liver, kidney and blood, and monitor clinical responses in this therapeutic trial.

CONTRACT NARRATIVE

DEVELOPMENTAL AND METABOLIC NEUROLOGY BRANCH
DIVISION OF INTRAMURAL RESEARCH, NINDS
OCTOBER 1, 1991 THROUGH SEPTEMBER 30, 1992

Contractor: GENZYME CORPORATION, BOSTON, MA. (N01-NS-9-2360)

Title: Preparation of Sphingomyelinase and Beta-Galactosidase from Human Placental Tissue

Contractor's Project Director: F. Scott Furbish

This contract was terminated as of July 1992.

CONTRACT NARRATIVE

DEVELOPMENTAL AND METABOLIC NEUROLOGY BRANCH
DIVISION OF INTRAMURAL RESEARCH, NINDS
JULY 16, 1990 THROUGH SEPTEMBER 30, 1991

Contractor: UNIVERSITY OF NEW MEXICO (NO1-NS-0-2394)

Title: Preparation of Homogeneous Human Liver Sterol Carrier Protein-2
(SCP-2) And Production of High-Titer Antibody TO SCP-2

Contractor's Project Director: Terence J. Scallen, M.D., PH.D.

This contract has been terminated as of July 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS00815-32DMN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism of Complex Lipids of Nervous Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P.G. Pentchev, Ph.D.	Section Chief	DMN	NINDS
Others: R.O. Brady, M.D.	Chief	DMN	NINDS
J.M. Quirk, M.S.	Biochemist	DMN	NINDS
C. Roff, Ph.D.	Special Expert	DMN	NINDS
E. Goldin, Ph.D.	Visiting Fellow	DMN	NINDS
M. Comly, B.S.	Biologist	DMN	NINDS
A. Cooney, B.S.	Biologist	DMN	NINDS

COOPERATING UNITS (if any)

Laboratory of Cellular and Developmental Biology, NIDDK, Laboratory of Biochemistry, Faculty of Medicine, Lyon-Sud, France

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Enzymology and Genetics, Molecular and Cellular Pathophysiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

8

PROFESSIONAL:

6.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The metabolic defect in patients with Types C and D Niemann-Pick disease has been shown to be due to abnormal intracellular cholesterol homeostasis. The molecular lesion in these disorders results in: (1) failure to down-regulate LDL receptors on cell membranes; (2) lack of down-regulation of HMGCoA reductase, a key enzyme in cholesterol biosynthesis; and (3) inability to up-regulate acyl cholesterol acyl CoA transferase, the enzyme that catalyzes the esterification of intracellular cholesterol. Tests have been developed and introduced into medical practice for the diagnosis of Types C and D Niemann-Pick disease and the identification of heterozygotes, and the prenatal diagnosis of these conditions.

We have observed that the addition of progesterone to cultured skin fibroblasts derived from normal individuals reversibly blocks the intracellular translocation of cholesterol that is similar to that in cells from patients with Type C Niemann-Pick disease (NPC). We also found that naturally occurring organic amines such as stearylamine and sphingosine elicit effects that closely mimic the abnormal cell biology of NPC. Moreover, we discovered that the quantity of sphinganine, a precursor of sphingosine, is greatly increased in the liver of the murine analogue of human NPC. These findings provide considerable additional insight into the pathogenesis of this neurometabolic disorder.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01NS02162-18DMN									
PERIOD COVERED October 1, 1991 to September 30, 1992											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Synthesis of Compounds Analogous to Glycolipids											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: S.P. Miller, Ph.D.</td> <td style="width: 33%;">Special Expert</td> <td style="width: 33%;">DMN NINDS</td> </tr> <tr> <td>Others: A. Boumendjel, Ph.D.</td> <td>Visiting Fellow</td> <td>DMN NINDS</td> </tr> <tr> <td>C. R. Kaneshi, B.S.</td> <td>Biologist</td> <td>DMN NINDS</td> </tr> </table>			PI: S.P. Miller, Ph.D.	Special Expert	DMN NINDS	Others: A. Boumendjel, Ph.D.	Visiting Fellow	DMN NINDS	C. R. Kaneshi, B.S.	Biologist	DMN NINDS
PI: S.P. Miller, Ph.D.	Special Expert	DMN NINDS									
Others: A. Boumendjel, Ph.D.	Visiting Fellow	DMN NINDS									
C. R. Kaneshi, B.S.	Biologist	DMN NINDS									
COOPERATING UNITS (if any)											
LAB/BRANCH Developmental and Metabolic Neurology											
SECTION Neurochemical Methodology Section											
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD. 20892											
TOTAL STAFF YEARS: <div style="text-align: center;">1.2</div>	PROFESSIONAL: <div style="text-align: center;">1.1</div>	OTHER: <div style="text-align: center;">0.1</div>									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </td> <td style="width: 33%; vertical-align: top;"> <input checked="" type="checkbox"/> (b) Human tissues </td> <td style="width: 33%; vertical-align: top;"> <input type="checkbox"/> (c) Neither </td> </tr> </table>			<input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither						
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project covers the synthesis of enzyme <u>substrates</u> and enzyme <u>inhibitors</u> which are used to study <u>sphingolipid metabolism</u>. The major ongoing project is the design and <u>synthesis of inhibitors of sphingosine-1-phosphate lyase</u>. This enzyme catalyzes the last step in the degradation of sphingosine: the cleavage of sphingosine phosphate to palmitaldehyde and ethanolamine phosphate. Very little is known about this enzyme and about the role(s) of free sphingosine <i>in vivo</i>. Inhibitors of sphingosine-1-phosphate lyase could advance understanding sphingosine catabolism in two ways. The preparation of radiolabeled irreversible inhibitors would aid in the isolation and purification of the enzyme. This could lead to partial sequence determination, and ultimately to cloning of the human or mouse gene. No sequence data are currently available on sphingosine-1-phosphate from any organism. A second use for enzyme inhibitors would be to provide information on the biological effects of blocking sphingosine catabolism <i>in vivo</i>. Sphingosine has been reported to be an inhibitor of protein kinase C, and sphingosine-1-phosphate causes rapid translocation of calcium from intracellular stores. Blocking sphingosine-1-phosphate lyase would lead to accumulation of these two compounds, possibly causing profound changes in cellular regulation. </p> <p> Our approach to the design of inhibitors is based upon the fact that sphingosine-1-phosphate lyase is a pyridoxal phosphate-dependent enzyme. Three initial synthetic targets were chosen for synthesis. They are the 2-difluoromethyl and the 2-vinyl derivatives of dihydrosphingosine, and 1,3-dihydroxy-2-hydrazino-octadecane. Progress has been made on all three syntheses, and small samples of the hydrazine analog are now available for characterization and testing. Initial assays will use <i>Tetrahymena furgasoni</i> as the enzyme source. The degradation of sphingosine in <i>Tetrahymena</i> occurs by the same two-step pathway (via sphingosine kinase and sphingosine-1-phosphate lyase) that is utilized by mammals. We are now culturing this organism in our laboratory, and are developing a bioassay for sphingosine catabolism that can be used to screen inhibitors. High specific activity [³H]-dihydrosphingosine that was previously synthesized in this laboratory is being used in these bioassays. Initial studies will follow sphingosine catabolism in whole cells of this protozoan. Subsequently studies will use sphingosine-1-phosphate lyase that will be isolated from <i>Tetrahymena</i> and mammalian sources. </p>											
11 DMN/DIR											

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01N502163-18DMN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Analytical Methods for Use in Research on Sphingolipidoses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. P. Miller, Ph.D.

Special Expert

DMN NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Neurochemical Methodology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

New analytical techniques were developed and used in enzymatic research and in clinical investigations of lipidoses. Gaucher's disease is a lipidosis caused by a deficiency of the lysosomal enzyme, glucocerebrosidase. Significant changes occur in the bone marrow of patients with this disease. In order to understand the biochemical basis of these changes, a study of the lipid components of normal and Gaucher's bone marrow was continued. A pronounced drop in the triglyceride level of Gaucher marrow (51 ± 53 mg/g wet weight) relative to normal controls (278 ± 70 mg/g wet weight) is by far the greatest change of all lipids on a weight basis. This decrease greatly overshadows the increase in glucocerebroside, and leads to an overall decrease in marrow lipids in Gaucher's disease. This explains the decrease in fat fraction that has been previously seen in magnetic resonance imaging studies of bone marrow in patients with Gaucher's disease. It is probable that these changes are due to the displacement of marrow adipocytes by infiltrating Gaucher macrophages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01NS02453-12DMN																														
PERIOD COVERED October 1, 1991 to September 30, 1992																																
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gaucher's Disease: Biochemical and Clinical Studies																																
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">N. Barton, M.D., Ph.D.</td> <td style="width: 25%;">Chief, Clinical Care Unit</td> <td style="width: 10%;">DMN</td> <td style="width: 10%;">NINDS</td> </tr> <tr> <td>OTHERS:</td> <td>R. O. Brady, M.D.</td> <td>Chief</td> <td>DMN</td> <td>NINDS</td> </tr> <tr> <td></td> <td>G. Murray, Ph.D.</td> <td>Special Volunteer</td> <td>DMN</td> <td>NINDS</td> </tr> <tr> <td></td> <td>G. Zirzow, B.S.</td> <td>Biologist</td> <td>DMN</td> <td>NINDS</td> </tr> <tr> <td></td> <td>K. Oliver, M.S.</td> <td>Biologist</td> <td>DMN</td> <td>NINDS</td> </tr> <tr> <td></td> <td>F. S. Jin, M.D.</td> <td>Special Volunteer</td> <td>DMN</td> <td>NINDS</td> </tr> </table>			PI:	N. Barton, M.D., Ph.D.	Chief, Clinical Care Unit	DMN	NINDS	OTHERS:	R. O. Brady, M.D.	Chief	DMN	NINDS		G. Murray, Ph.D.	Special Volunteer	DMN	NINDS		G. Zirzow, B.S.	Biologist	DMN	NINDS		K. Oliver, M.S.	Biologist	DMN	NINDS		F. S. Jin, M.D.	Special Volunteer	DMN	NINDS
PI:	N. Barton, M.D., Ph.D.	Chief, Clinical Care Unit	DMN	NINDS																												
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	K. Oliver, M.S.	Biologist	DMN	NINDS																												
	F. S. Jin, M.D.	Special Volunteer	DMN	NINDS																												
COOPERATING UNITS (if any) Massachusetts Gen. Hospital, Dept. of Orthopedic Surgery, Boston, MA: (H. Mankin, D. Rosenthal, S. Doppelt); Children's Hospital, Washington, D. C. (P. Guzzetta)																																
LAB/BRANCH Developmental and Metabolic Neurology																																
SECTION Clinical Investigations & Therapeutics Section																																
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892																																
TOTAL STAFF YEARS: <div style="text-align: center; font-weight: bold;">5.5</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">3.5</div>	OTHER: <div style="text-align: center; font-weight: bold;">2.0</div>																														
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"><input type="checkbox"/> (a) Human subjects</td> <td style="width: 33%;"><input checked="" type="checkbox"/> (b) Human tissues</td> <td style="width: 33%;"><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews																							
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Extraordinarily gratifying success has been obtained with <u>enzyme replacement therapy</u> in patients with <u>Gaucher's disease</u> . All patients who received <u>macrophage-targeted</u> human placental <u>glucocerebrosidase</u> had significant clinical benefit. The hemoglobin level rose in all patients, and within six months after initiation of therapy, the size of the spleen had decreased in all recipients. The enzyme injections were well tolerated, and none of the patients became sensitized to the preparation. Patients who received the enzyme were able to resume activities such as work or school that they had been unable to carry out before enzyme replacement. The U.S. Food and Drug Administration has approved the use of macrophage-targeted glucocerebrosidase as specific therapy for patients with Type 1 Gaucher's disease. The beneficial effect of enzyme replacement in patients with Gaucher's disease has been repeatedly confirmed by many independent investigators. We have found that the quantity of enzyme that patients require to be maintained in good health is far less than that which is initially necessary to reverse the clinical and pathologic manifestation of the disorder. Patients with milder clinical signs of the disorder improve with smaller amounts of enzyme than that required by more severely affected individuals.																																

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01N502664-08DMN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Neurogenetic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. Barton, M.D., Ph.D.	Section Chief	DMN	NINDS
OTHERS: R. Brady, M.D.	Chief	DMN	NINDS
J. Higgins, M.D.	Clinical Associate	DMN	NINDS
M. Patterson, M.D.	Visiting Associate	DMN	NINDS
C. Parker, M.D.	Clinical Associate	DMN	NINDS
R. Schiffmann, M.D.	Clinical Associate	DMN	NINDS

COOPERATING UNITS (if any)

Neuroimaging Branch, NINDS, and Laboratory of Molecular and Cellular Neurobiology, NINDS

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF YEARS:

6.0

PROFESSIONAL:

6.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The clinical staging of the progression of neurologic signs in patients with Type C Niemann-Pick disease has been carefully documented. We are using this paradigm to assess the effect of cholesterol-lowering agents on these individuals since the intraneuronal accumulation of this lipid is a hallmark of the disorder. We have identified a new demyelinating disorder in young females and documented highly unusual magnetic resonance spectroscopic aberrations in these patients.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01N502731-06DMN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Therapy of Inherited Enzyme Deficiencies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Karlsson, M.D., Ph.D.	Acting Chief, M&MG	DMN	NINDS
OTHERS:	L. Xu, M.D., Ph.D.	Visiting Associate	DMN	NINDS
	H. Dave, M.D.	Visiting Associate	DMN	NINDS
	S. Colilla, B.S.	Special Volunteer	DMN	NINDS
	P. Correll, B.S.	Special Volunteer	DMN	NINDS
	R. Brady, M.D.	Chief	DMN	NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Molecular and Medical Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

12.0

PROFESSIONAL:

6.5

OTHER:

5.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Gaucher's disease is an inherited disorder caused by a mutation of the gene for the enzyme glucocerebrosidase. The normal gene for this enzyme has been cloned by several laboratories. We have constructed high-titer, helper-free recombinant retroviruses containing this gene. We have shown that infection of cell lines from normal individuals and patients with Gaucher's disease with this retroviral vector results in increased glucocerebrosidase activity. The glucocerebrosidase gene has been transferred efficiently into progenitor cells and repopulating stem cells of mouse bone marrow, and is expressed at the RNA and protein level in the progeny of CFU-S multipotential progenitor cells following gene transfer. The gene has also been transferred efficiently into murine hematopoietic stem cells that can be used to repopulate secondary transplant recipients. The vector genome can be detected in all hematopoietic lineages and produces human glucocerebrosidase RNA in all hematopoietic tissues tested. High levels of human glucocerebrosidase are generated in hematopoietic tissues. The macrophages of these long-term reconstituted mice produce human glucocerebrosidase levels that are equivalent to the endogenous mouse enzyme levels. The human glucocerebrosidase gene has been introduced into human hematopoietic progenitor cells with a high-degree of efficiency. Vector-transduced hematopoietic progenitors from Gaucher's patients produce progeny cells with glucocerebrosidase enzyme values similar to those of normal individuals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS02769-04DMN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Exploration of Strategies for the Treatment of AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R.R. O'Neill, Ph.D.	Senior Staff Fellow	DMN	NINDS
OTHERS: E. Carstea, Ph.D.	Staff Fellow	DMN	NINDS
H. Christakis, B.S.	Biologist	DMN	NINDS
S. Miller, Ph.D.	Special Expert	DMN	NINDS
R.O. Brady, M.D.	Chief	DMN	NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Enzymology and Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

0.7

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project terminated. Investigator left NIH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS02771-04DMN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Modification of Growth Factor Genes by Gene Targeting

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Kulkarni, Ph.D.	Senior Staff Fellow	DMN	NINDS
OTHERS: S. Karlsson, M.D., Ph.D.	Acting Section Chief	DMN	NINDS
D. Becker, B. S.	Biologist	DMN	NINDS
C.-G. Huh, Ph.D.	IRTA Fellow	DMN	NINDS
M. Lyght, B. S.	Biologist	DMN	NINDS

COOPERATING UNITS (if any)

A. Geiser, Ph.D., Biologist, Laboratory of Chemoprevention, NCI

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular and Medical Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF-YEARS:

5.5

PROFESSIONAL:

3.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gene targeting by homologous recombination can be used to activate or inactivate cellular genes in eukaryotic cells. The objective of this project is to alter the functional status of genes that control growth and maturation of specific tissues and study the biological consequences of these molecularly defined alterations. The tumor growth factor beta (TGF-beta) has been chosen for this study as it is a well-characterized gene whose biological functions are well known. The biological importance of the TGF-beta gene will be studied in the context of a whole organism by targeting a defective gene to its cognate counterpart in embryonic stem (ES) cells by homologous recombination. These cells will subsequently be used to generate transgenic mice containing a defective TGF beta 1 gene. The TGF-beta 1 gene has been targeted in ES cells. Six targeted clones have been generated and four of these have been used to generate chimeric mice. Two of the clones could generate germ-line chimeras. The offspring of these chimeras carry a mutated TGF beta 1 gene. These heterozygous mice are normal and have been mated to yield mice homozygous for the null TGF beta 1 allele. Two homozygous mice have been born. They are runted, have difficulty opening their eyelids, and die at 3 weeks. Study of this pathological condition is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01NS02782-03DMN																		
PERIOD COVERED October 1, 1991 through September 30, 1992																				
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Preparation of Transgenic Murine Analogs of Human Metabolic Storage Disorders																				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: R.R. O'Neill, Ph.D.</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">DMN NINDS</td> </tr> <tr> <td>Others: E. Carstea, Ph.D.</td> <td>Staff Fellow</td> <td>DMN NINDS</td> </tr> <tr> <td>H. Christakis, B.S.</td> <td>Biologist</td> <td>DMN NINDS</td> </tr> <tr> <td>S. Karlsson, M.D., Ph.D.</td> <td>Acting Chief</td> <td>DMN NINDS</td> </tr> <tr> <td>A. Kulkarni, Ph.D.</td> <td>Senior Staff Fellow</td> <td>DMN NINDS</td> </tr> <tr> <td>R. Brady, M.D.</td> <td>Chief</td> <td>DMN NINDS</td> </tr> </table>			PI: R.R. O'Neill, Ph.D.	Senior Staff Fellow	DMN NINDS	Others: E. Carstea, Ph.D.	Staff Fellow	DMN NINDS	H. Christakis, B.S.	Biologist	DMN NINDS	S. Karlsson, M.D., Ph.D.	Acting Chief	DMN NINDS	A. Kulkarni, Ph.D.	Senior Staff Fellow	DMN NINDS	R. Brady, M.D.	Chief	DMN NINDS
PI: R.R. O'Neill, Ph.D.	Senior Staff Fellow	DMN NINDS																		
Others: E. Carstea, Ph.D.	Staff Fellow	DMN NINDS																		
H. Christakis, B.S.	Biologist	DMN NINDS																		
S. Karlsson, M.D., Ph.D.	Acting Chief	DMN NINDS																		
A. Kulkarni, Ph.D.	Senior Staff Fellow	DMN NINDS																		
R. Brady, M.D.	Chief	DMN NINDS																		
COOPERATING UNITS (if any)																				
LAB/BRANCH Developmental and Metabolic Neurology																				
SECTION Enzymology and Genetics																				
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD. 20892																				
TOTAL MAN-YEARS: <div style="text-align: center;">1.5</div>	PROFESSIONAL: <div style="text-align: center;">1.2</div>	OTHER: <div style="text-align: center;">0.3</div>																		
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews											
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither																		
<input type="checkbox"/> (a1) Minors																				
<input type="checkbox"/> (a2) Interviews																				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Project terminated. Investigator left NIH.																				

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS02785-04DMN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Generation of Mice with Sickle Cell Anemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Karlsson, M.D., Ph.D.	Acting Chief, M&MG	DMN	NINDS
OTHERS:	D. Freas, B.S.	Chemist	DMN	NINDS
	A. Schechter, M.D.	Chief,	LCB	NINDS
	C. Noguchi, Ph.D.	Sen. Scientist	LCB	NIDDK
	F. Shafer, M.D.	Staff Fellow	LCB	NIDDK

COOPERATING UNITS (if any)

Dept. of Biology, Univ. of S. Carolina, Prof. M. Dewey

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular and Medical Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF MAN-YEARS:

4.0

PROFESSIONAL:

2.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Transgenic mouse technology can be utilized to produce animals expressing a foreign gene to high levels of its RNA and protein. The objective of this project is to generate mice that contain an abnormal beta-globin gene (beta-sickle Antilles). This gene has two mutations and generates sickle cell anemia in a heterozygous individual. High level expression of the gene is obtained by inserting the dominant control region from the human beta globin locus in cis to the Antilles gene. This dominant control region allows high level globin expression to occur. The human alpha-globin gene is also inserted in cis in order to generate mice that can produce high levels of beta sickle Antilles and human alpha globins. Three independent transgenic mouse lines that express both human globin genes have been generated. Expression levels of human alpha globin RNA are high but levels of the human beta sickle Antilles RNA have been consistently low. Homozygous mice with these constructs cannot be generated easily and there is a genetic evidence that the beta sickle Antilles gene may generate harmful effects during development of the mouse. Due to imbalance of alpha and beta globin chains there is increased fetal demise even in heterozygous animals. This effect is more pronounced when the transgenics are bred on a background of beta thalassemic mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01N502816-03DMN

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis of Inhibitors of N-Myristoyltransferase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. P. Miller, Ph.D	Special Expert	DMN	NINDS
Others: K.M. Neder, Ph.D	IRTA Fellow	DMN	NINDS
S. A. French, B.S.	Chemist	DMN	NINDS
R. R. O'Neill, Ph.D.	Sen. Staff Fellow	DMN	NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Neurochemical Methodology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF-YEARS:

2.7

PROFESSIONAL:

1.7

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The goal of this project is the synthesis of new inhibitors of the enzyme myristoyl-CoA:protein N-myristoyltransferase (NMT). This enzyme catalyzes the covalent modification of specific proteins by acylating the amino group of N-terminal glycines with myristoyl-CoA. Biomedically important proteins which are myristoylated include oncoproteins such as p60 src and the gag polypeptide of HIV and other retroviruses. The goal of this project is to design and synthesize inhibitors of protein myristoylation that are active *in vivo* for testing as antiretroviral agents. More than 35 analogs of the substrate, myristoyl-CoA, or of the myristoylated peptide product have been synthesized. These include compounds designed to be either competitive or irreversible inhibitors of NMT. All have been tested in an *in vitro* NMT assay developed within this project. The regions of the substrates and products that are necessary for high-affinity binding to NMT are being identified and incorporated into future syntheses. One synthetic compound has shown activity in the renal cancer panel of the NCI *In Vitro* Primary Antitumor Screen. It has been selected by the NCI Biological Evaluation Committee for further testing in mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01NS02843-01DMN												
PERIOD COVERED October 1, 1991 to September 30, 1992														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Investigation of the Etiology Mucopolipidoses IV.														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: R.O. Brady, M.D.</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">DMN NINDS</td> </tr> <tr> <td>Others: E. Goldin, Ph.D.</td> <td>Visiting Fellow</td> <td>DMN NINDS</td> </tr> <tr> <td>P.G. Pentchev, Ph.D.</td> <td>Section Chief</td> <td>DMN NINDS</td> </tr> <tr> <td>N.W. Barton, M.D., Ph.D.</td> <td>Section Chief</td> <td>DMN NINDS</td> </tr> </table>			PI: R.O. Brady, M.D.	Chief	DMN NINDS	Others: E. Goldin, Ph.D.	Visiting Fellow	DMN NINDS	P.G. Pentchev, Ph.D.	Section Chief	DMN NINDS	N.W. Barton, M.D., Ph.D.	Section Chief	DMN NINDS
PI: R.O. Brady, M.D.	Chief	DMN NINDS												
Others: E. Goldin, Ph.D.	Visiting Fellow	DMN NINDS												
P.G. Pentchev, Ph.D.	Section Chief	DMN NINDS												
N.W. Barton, M.D., Ph.D.	Section Chief	DMN NINDS												
COOPERATING UNITS (if any)														
LAB/BRANCH Developmental and Metabolic Neurology														
SECTION Cellular and Molecular Pathophysiology														
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892														
TOTAL STAFF YEARS: <div style="text-align: center;">1.2</div>	PROFESSIONAL: <div style="text-align: center;">1.2</div>	OTHER:												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"><input type="checkbox"/> (a) Human subjects</td> <td style="width: 33%;"><input checked="" type="checkbox"/> (b) Human tissues</td> <td style="width: 33%;"><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews					
<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither												
<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The etiology of <u>Mucopolipidosis</u> IV is currently unknown. We shall explore rational biochemical and cell biology leads to obtain insight into the pathogenesis and molecular abnormality in this hereditary disorder. Our principal goals are to develop accurate diagnostic and carrier detection tests for genetic counseling and realistic approaches to the therapy of this hereditary disorder.														

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS02844-01DMN

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Investigation of the Etiology of Batten's disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Calvin F. Roff, Ph.D.

Others: Peter G. Pentchev, Ph.D.

Roscoe O. Brady, M.D.

Section Chief

Branch Chief

DMN

DMN

NINDS

NINDS

COOPERATING UNITS (if any)

Section on Receptor Biochemistry and Molecular Biology, NINDS

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular and Cellular Pathophysiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

TOTAL STAFF-YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

-

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The etiology of Batten's disease is unknown at this time. We intend to explore biochemical cell biological and molecular biological leads to other information on the pathogenesis and molecular abnormalities in this and closely related conditions. Our goals are to develop precise diagnostic tests and effective therapies for patients with these disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01NS02845-01DMN									
PERIOD COVERED October 1, 1991 to September 30, 1992											
TITLE OF PROJECT <small>(80 characters or less. Title must fit on one line between the borders.)</small> Investigation of Enzyme Replacement Therapy in an Analogue of Human GM1-Gangliosidosis											
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small> <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: R. O. Brady, M.D.</td> <td style="width: 30%;">Chief</td> <td style="width: 30%;">DMN NINDS</td> </tr> <tr> <td>Others: G.J. Murray, Ph.D.</td> <td>Special Volunteer</td> <td>DMN NINDS</td> </tr> <tr> <td>J.M. Quirk, M.S.</td> <td>Biochemist</td> <td>DMN NINDS</td> </tr> </table>			PI: R. O. Brady, M.D.	Chief	DMN NINDS	Others: G.J. Murray, Ph.D.	Special Volunteer	DMN NINDS	J.M. Quirk, M.S.	Biochemist	DMN NINDS
PI: R. O. Brady, M.D.	Chief	DMN NINDS									
Others: G.J. Murray, Ph.D.	Special Volunteer	DMN NINDS									
J.M. Quirk, M.S.	Biochemist	DMN NINDS									
COOPERATING UNITS <small>(if any)</small> Surgical Neurology Branch, NINDS											
LAB/BRANCH Developmental and Metabolic Neurology Branch											
SECTION Enzymology and Genetics											
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892											
TOTAL STAFF-YEARS: <div style="text-align: center;">2.0</div>	PROFESSIONAL: <div style="text-align: center;">1.5</div>	OTHER: <div style="text-align: center;">0.5</div>									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"><input type="checkbox"/> (a) Human subjects</td> <td style="width: 33%; text-align: center;">(b) Human tissues</td> <td style="width: 33%; text-align: center;"><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	(b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews		
<input type="checkbox"/> (a) Human subjects	(b) Human tissues	<input checked="" type="checkbox"/> (c) Neither									
<input type="checkbox"/> (a1) Minors											
<input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided.)</small> <u>Enzyme replacement therapy</u> has been shown to be extraordinarily effective for patients with <u>Type 1 (non-neuronopathic) Gaucher's disease</u> . We now need to develop procedures to deliver useful amounts of enzymes to the brain in patients with hereditary metabolic storage disorders. Using a new intracerebral protein delivery system. We shall examine the effect of human placental beta-galactosidase on the amount of ganglioside GM1 in animal analogues of human generalized (GM1) gangliosidosis.											

ANNUAL REPORT

**October 1, 1992 through September 30, 1992
Experimental Therapeutics Branch
Clinical Neurosciences Program, DIR
National Institute of Neurological Disorders and Stroke**

TABLE OF CONTENTS

RESEARCH SUMMARY	1-29
PROJECT REPORTS	
Biochemical and Pharmacological Studies of Dopamine Receptors Z01 NS-02263-16 ET	30
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Pharmacology, Biochemistry and Physiology of Central Neurotransmitters Z01 NS-02265-16 ET	33

ANNUAL REPORT

October 1, 1990 through September 31, 1991

Experimental Therapeutics Branch
Clinical Neurosciences Program, DIR
National Institute of Neurological Disorders and Stroke
Thomas N. Chase, M.D., Chief

The Experimental Therapeutics Branch (ETB) has as its goal the development of improved pharmacotherapies for neurologic disease. To this end, the Branch operates a vertically integrated program of research extending from basic neurobiology to clinical trials. These investigative efforts continue to focus on neurodegenerative disorders that impair motor and cognitive function.

ETB is organized into four highly interactive operating components: 1) The Molecular Neuropharmacology Section, directed by Dr. David Sibley, conducts molecular and biochemical investigations to characterize central transmitter receptors and information transduction processes; 2) The Genetic Pharmacology Unit, led by Dr. Maral Mouradian, seeks at the molecular level to develop pharmaceutical approaches to the selective regulation of gene expression within the mammalian central nervous system(cns); 3) The Neurophysiologic Pharmacology Section, operating at the neuronal network level under the leadership of Dr. Judith Walters, studies basal ganglia function especially in relation to dopamine receptor mechanisms and the effect of drugs that influence motor behavior; and 4) The Clinical Pharmacology Section under Dr. Thomas Chase works with neurologic patients and in animal models to elucidate pathophysiologic mechanisms and develop novel pharmaceutical interventions.

Molecular Neuropharmacology Section

The Molecular Neuropharmacology Section continued investigating the biochemical, molecular, and pharmacologic properties of dopamine (DA) and other neurotransmitter receptors in FY92. The long-term goal of the Section is the characterization of neurotransmitter receptor-mediated information transduction, and its regulation, across neuronal membranes. DA receptors are used primarily, but not exclusively, as representative model systems for the large class of neurotransmitter receptors which are linked to their biochemical effectors via guanine nucleotide binding regulatory (G) proteins. In order to characterize the DA and other neurotransmitter receptors at the biochemical and molecular levels and study their regulation, there are two major ongoing interrelated lines of research: 1) investigation of the cell biology, function and regulation of the receptors at the protein level; and 2) molecular cloning of the receptor genes and investigation of gene structure and regulation in normal and pathophysiologic states.

1. Cell Biology and Regulation of DA Receptors.

The objective of this line of inquiry is to obtain a detailed understanding of signal transduction and regulation of dopamine receptor function. Our efforts in FY92 have been to continue to capitalize on our discovery of various mammalian cell lines that express either D₁ or D₂ receptors and conduct studies of DA receptor regulatory mechanisms. In FY92, we began to

investigate the regulation of cloned D₁ and D₂ receptors which are expressed in stably transfected cell lines.

We have further investigated D₁ receptor regulation in the NS20Y cells, which endogenously express this receptor subtype, with particular emphasis on determining potential feedback mechanisms involving the second messenger cAMP. Treatment of the cells with 8-(4-chlorophenylthio)-adenosine-3':5'-cyclic monophosphate (CPT-cAMP), a membrane-permeable analog of cAMP, induced desensitization of D₁ receptor-coupled adenylyl cyclase (AC) activity. CPT-cAMP treatment also induced down-regulation of D₁ receptor binding activity, the time course and extent of which matched (AC) inactivation. These data suggest that cAMP mediates desensitization of the D₁ receptor involving functional uncoupling and receptor down-regulation. We have related this to the DA-induced desensitization by investigating what aspects of the DA-mediated regulation response are blocked by the cAMP antagonist, Rp-cAMPs. We found that although Rp-cAMPs did not block the maximal desensitization/down-regulation induced by DA, recovery to basal levels of activity was faster in the presence of Rp-cAMPs. This suggests that certain aspects of the DA-induced desensitization of the D₁ receptor is definitively mediated by cAMP operating through a classic negative feedback loop. The precise mechanism(s) of the cAMP-mediated desensitization as well as what other mechanisms are operative are being further investigated.

To further investigate the functional and regulatory properties of the D_{1A} receptor, we have stably expressed a cDNA encoding that rat D_{1A} receptor in Chinese hamster ovary (CHO) cells. Pretreatment of these cells with DA profoundly desensitized the DA-stimulated cAMP response and a loss of [³H]-SCH-23390 binding. The DA-induced desensitization/down-regulation was time-dependent, reaching maximal levels after 20 hr with a $t_{1/2} > 5$ hr. The DA dose-response for promoting these regulatory effects shows an EC₅₀ of about 10 nM with an EC_{max} of 10 μ M. After maximal desensitization, the EC₅₀ of DA for stimulating cAMP accumulation is unchanged whereas the maximum response is decreased by about 75-90%. Similarly, there is no change in the affinity (K_p) of [³H]-SCH-23390 but a >50% decrease in maximum binding capacity is observed. The agonist-induced desensitization is pharmacologically-specific being mimicked by 6,7-ADTN, fenoldopam, and SKF38393. We are currently investigating the underlying biochemical and molecular mechanisms associated with these processes with particular emphasis on determining if covalent modification, such as phosphorylation of the receptors is involved.

In order to facilitate experimentation on the biochemical mechanisms of D₂ receptor regulation, we have stably transfected CHO cells with the cloned D₂ receptor (both short and long isoforms) in FY92 in order to create high-expressing cell lines. A number of cell lines were produced expressing various levels of D₂ receptor protein. Initial experiments in FY92 were directed at examining the regulatory properties of the rat D_{2L} DA receptor expressed in the CHO cells. Pretreatment of these cells with DA produced a 5-fold shift (to lower affinity) in the EC₅₀ for DA inhibition of cAMP accumulation but no change in the maximum response. Surprisingly, the DA pretreatment resulted in a 3 fold increase in the maximum receptor binding capacity with no change in receptor affinity. This effect was time-dependent reaching maximal levels after 24 hr with a $t_{1/2} > 5$ hr. In a preliminary experiment, prior treatment of the cells with a pertussis toxin did not block the DA-induced increase in radioligand binding. Treatment of the cells with

various intracellular activators of protein kinases resulted in a general blunting of the D_{2L} receptor responses. Exposure to the phorbol ester and potent activator of protein kinase CPMA, resulted in a 3-fold reduction in the potency of DA to inhibit cAMP accumulation with no change in the maximum response along with a 25% decrease in receptor B_{max} values. Similarly, treatment with Sp-cAMPS for 24 hr resulted in a 25-50% reduction in the maximum DA cAMP response with no change in potency along with a 25-50% reduction in B_{max} values. We are currently investigating the underlying biochemical and molecular mechanisms associated with these processes with particular emphasis on determining if covalent modification, such as phosphorylation, is involved. Similar experiments involving the D_{2S} isoform are also currently being performed.

As it would be of interest to express the D₂ receptor in a neural cell type in order to examine receptor-mediated ion conductances, we have transfected the D₂ receptor in NG108-15 neuroblastoma cells. We were successful in obtaining several cell lines which express low levels (10-100 fmol/mg protein) of both short and long isoforms of the D₂ receptor. In collaboration with Drs. Greg Kapatos and Lou Chiodo at Sinai Hospital in Detroit, we have begun to investigate the electrophysiologic characteristics of the transfected cells. To begin such an analysis, NG108-15 cells which stably express the short receptor isoform (D_{2S}) were examined using the whole-cell patch-clamp technique. These cells were found to exhibit inward currents mediated by both T- and L-type Ca²⁺ channels as defined by voltage-dependence of activation, rate of inactivation and sensitivity to antagonists. Pressure application of DA or the D₂-selective agonist, quinpirole, reduced both T- and L-type currents. These agonist effects were blocked by cotreating the cells with the D₂-selective antagonist, eticlopride. Application of DA or quinpirole also reduced the amplitude of a K⁺-dependent outward current. This effect was blocked in a concentration-dependent manner by inclusion of the Ca²⁺ chelator BAPTA in the pipette solution, was not altered by Co²⁺ in the bath solution and could be mimicked by pressure application of thapsigargin. These results suggest that Ca²⁺ mobilized from intracellular stores is involved in the reduction of this K⁺ current by D_{2S} receptor stimulation. No effect of dopaminergic agonists on inward or outward currents was observed using untransfected cells. The inhibitory effect of D_{2S} receptor stimulation on two distinct Ca²⁺-dependent inward currents and a K⁺-dependent outward current suggests a common mechanism, possibly mediated by mobilization of intracellular Ca²⁺, may be involved. Similar studies of NG108-15 cells stably transfected with the D_{2L} isoform are currently in progress and should determine whether the actions of these two isoforms of the D₂ receptor are mediated by similar signal transduction mechanisms.

2. Molecular Cloning of DA and Other G Protein-Linked Receptors.

In FY92 we have continued our molecular biological characterization of the D_{1A} and D_{2L} receptors which were initially cloned by us as well as the D₃ receptor which we cloned by PCR. For the D₂ receptor, we have continued to investigate the potential functional significance of its two isoforms which were derived from alternative RNA splicing. To investigate the functional properties of the short (D_{2S}) and long (D_{2L}) isoforms of the D₂ receptor, we have stably expressed both rat cDNAs in CHO cells. Our investigations indicated that the D_{2L} and D_{2S} receptors are functionally similar in their

ligand binding properties and linkage to AC inhibition. We have also collaborated with Dr. Chris Felder in the Laboratory of Cell Biology, NIMH, to examine what role the D₂ receptor might play in the production of the second messenger, arachidonic acid. We demonstrated that both forms of the D₂ receptor were able to increase the release of arachidonic acid by a mechanism involving protein kinase C but independent of AC. Recent evidence from Dr. Gerry Oxford's laboratory has also indicated that both D₂ receptor isoforms can activate K⁺ channels in pituitary cells. Thus, all experiments to-date would argue for a functional similarity between the two isoforms. As mentioned above, in FY92 we have demonstrated that the longer D₂ receptor isoform will undergo agonist-induced desensitization along with a paradoxical up-regulation in our transfected CHO cells. We will test the shorter D₂ isoform to see if it exhibits similar or different regulatory properties. We will also examine both isoforms in terms of their regulation of ion channel activities in the NG108-15 neuroblastoma cell as discussed above.

In FY92, we have continued our molecular characterization of the D₁ (now the D_{1A}) DA receptor previously cloned by us. Primarily, we have created a number of transfected cell lines expressing various levels of receptor protein. These cells have been utilized to investigate the functional and regulatory characteristics of the D_{1A} receptor protein (see above). In order to further investigate the molecular biology of the D_{1A} and D₂ receptors, we have also isolated the genes from rat and human genomic libraries constructed in the EMBL3 vector. We have provided these to Dr. Maral Mouradian's Unit in ETB to characterize the regulatory elements in these genes.

As mentioned above, we have also cloned the rat D₃ receptor (which belongs to the D₂ receptor subcategory) using PCR. In addition to examining potential regulatory mechanisms for this receptor, we are particularly interested in examining its signal transduction pathway, which has not yet been identified. We have transfected a wide variety of cell lines with our D₃ receptor cDNA and plan to investigate potential linkage to AC, K⁺ and Ca²⁺ channel regulation as performed with the D₂ receptor splice variants. Particular emphasis is being placed on investigating the function of the D₃ receptor expressed in the NG108-15 neuroblastoma cells in collaboration with the group in Detroit. We are currently performing additional transfections with these cells to obtain lines with higher levels of expression.

We have used the rat kidney proximal convoluted tubule (PCT), which contains D₁ receptor subtypes linked to the activation of AC as well as phospholipase C, to investigate the molecular biology of peripherally located D₁ receptors. In order to clone these receptor subtypes, PCR was used to selectively amplify putative D₁ receptor cDNA sequences from rat kidney PCT mRNA. This process resulted in the amplification and cloning of a novel cDNA which exhibits considerable homology to dopaminergic and other members of the G protein-coupled receptor family. This rat receptor is 475 amino acids in length and is about 50% identical overall but >80% homologous in the transmembrane regions when compared to the previously cloned D₁ receptor. When compared to the recently cloned human "D₅" receptor, it is 83% identical overall but 95% identical in the transmembrane regions. Expression of the rat kidney D₁ receptor exhibits a similar pharmacology as the D₅ receptor including a relatively high affinity for and linkage to stimulating AC. Based on these data, we have concluded that the rat kidney D₁ receptor is the rat homolog of the human D₅ DA receptor. We, however, have designated this

second D₁ receptor to be cloned the "D_{1B}" receptor and now refer to the first D₁ receptor that we cloned as the "D_{1A}" receptor.

Using PCR amplification followed by Southern blotting of the cDNA products, we have demonstrated that both the D_{1A} and D_{1B} receptors are expressed in various kidney regions, including the inner and outer medulla, glomeruli and proximal convoluted tubules. In all regions examined, the D_{1B} receptor mRNA predominates over that for the D_{1A} receptor with both transcripts being expressed in highest abundance in the proximal convoluted tubules. We are currently using mRNA and antibody probes to further delineate the cellular distribution of both receptor subtypes in the kidney as well as evaluating their status in the spontaneously hypertensive rat (SHR) which exhibits defective D₁ receptor coupling in the kidney.

As part of our efforts to clone novel DA receptor subtypes, we have utilized PCR to selectively amplify G protein-coupled receptor cDNA sequences from rat striatal mRNA. One cDNA fragment was identified which exhibits high homology with previously cloned catecholamine receptors. Sequencing of a full-length clone, isolated from a rat striatal cDNA library using this fragment, revealed an open-reading frame of 1308 bp encoding a 436 residue protein with seven hydrophobic regions representing putative transmembrane spanning domains. Within these hydrophobic regions, this receptor was found to exhibit 44%-36% identity with the following receptors: D_{1A}=D_{1B}>D₂=D₃=D₄>5HT₂>5HT_{1C} 5HT_{1C}>5HT_{1Da}>5HT_{1A}>5HT_{1Db}. Northern blot analysis revealed a 3.8 kb transcript with the following rank order of abundance in CNS tissues: striatum >> olfactory tubercle > cortex > hippocampus. Expression of the full-length cDNA in COS-7 cells resulted in the appearance of high affinity and saturable [¹²⁵I]-LSD binding (K_D = 3 nM). Among all endogenous biogenic amines tested, only 5-HT completely inhibited [¹²⁵I]-LSD binding, exhibiting a K_i of = 100 nM. The rank order of potency for inhibition of [¹²⁵I]-LSD binding by other serotonergic agonists was: 5-MT>5-HT>5-CT>TFMPP>mCPP>>8-OH-DPAT. The rank order of antagonist binding was: mianserin >> (-) propranolol> SCH 23390> ketanserin = spiroperidol. As a group, ergoline derivatives displayed the highest affinities: lisuride>legotrile>bromocriptine>dihydroergotamine>2-Br-LSD>dihydroergocriptine. This pharmacologic profile does not correlate with any previously described 5-HT receptor subtype. The functional properties of this novel 5-HT receptor are currently under investigation.

In a related series of PCR experiments, we have cloned an additional novel 5-HT receptor cDNA which is distinct from the receptor discussed above. This sequence was initially identified as a cDNA fragment which was PCR-amplified from rat kidney proximal convoluted tubule mRNA. Northern blot analysis with this fragment reveals a -3.6 kb transcript with the following rank order of abundance in CNS tissues: hypothalamus>hippocampus = mesencephalon > olfactory bulb = cerebral cortex > olfactory tubercle > striatum. In peripheral tissues, this mRNA is most abundant in the spleen. In situ hybridization analysis confirms the Northern blot data and also reveals a high level of transcript in the thalamic reticular nucleus. Isolation and sequencing of a full-length clone obtained from a rat hippocampal library revealed a long open-reading frame encoding a 404 residue protein, the sequence of which is most closely related to the 5-HT receptor family. Within the 7 putative transmembrane spanning regions, this receptor exhibits 60% - 43% identity with the following receptors:

5HTDRO1>5HT1D>5HT1A>D1A>D2>5HT2>5HT1C. Expression of the full-length cDNA in COS cells resulted in the appearance of high affinity and saturable [³H]-LSD binding (K_D =5 nM). Among all endogenous biogenic amines tested, only 5-HT completely inhibited [³H]-LSD binding, exhibiting a K_i of 15 nM. The rank order of potency for inhibition of [³H]-LSD binding by other serotonergic agonists was: 5-CT>5-MT>5-CT>5HT>8-OH-DPAT>mCPP>TFMPP. The rank order of antagonist binding was: methiothepin>metergoline>mesulergine>methylsergide>mianserin>ketanserin. This pharmacologic profile does not appear to correlate with any previously described 5-HT receptor subtype including the one discussed above which we cloned from the rat striatum. The functional properties of this novel 5-HT receptor are also currently under investigation.

Genetic Pharmacology Unit

1. Transcriptional regulation of the D1A receptor gene:

Studies aimed at investigating the molecular regulation of the human D1A receptor gene were initiated at the end of FY90. A cDNA clone coding for the rat striatal AC-linked D1A receptor was used as a probe to screen a human genomic library. Of the positive clones isolated, #one (HDIG) had a 5.3 kb Sac I fragment which gave the strongest signal on Southern blot. During FY91, this DNA fragment was subcloned into pGEM-3Zf(-) and characterized. The 3.0 kb Pst I-Sac I fragment of HDIG, which was determined to represent the transcribed region of the gene, was excised and the plasmid religated, leaving a 2.3 Sac I-Pst I fragment insert, and giving the plasmid pDIG3. Both strands of this insert were completely sequenced.

Transcriptional initiation site of the D1A gene was determined initially with S1 nuclease analysis. For these experiments, RNA was isolated from postmortem human caudate tissue and a 5' end-labeled, single-stranded genomic DNA probe was prepared from pDIG3. Following hybridization of the probe with RNA, the reaction was subjected to S1 nuclease digestion and the protected fragment electrophoresed in a denaturing gel parallel to a DNA sequencing reaction using the same oligonucleotide primer that was used for generating the S1 nuclease probe. The procedure was repeated several times using different primers at different locations all yielding a single band, suggesting that transcription perhaps starts at nucleotide -486 relative to translation initiation site. However, the sequence in the immediate vicinity of this nucleotide had high homology to the splice acceptor consensus sequence. Furthermore, sequences homologous to a splice donor site were found at bases -549, -599 and -909. Thus, S1 mapping using a 5'-end labeled probe appears to have detected the 5' end of an exon. The possibility of the presence of an intron in the 5' untranslated region of the human D1A gene was pursued vigorously during FY92. To verify the intron/exon structure of this gene, a series of RT-PCR experiments were conducted using human caudate poly(A)+RNA and six different primers designed around these intron donor and acceptor sites, and used with a single 3' primer located at base -408 to -426. The results of these experiments collectively suggested that the human D1A gene has indeed a 116 bp long intron in its 5' untranslated portion. The presence of exon 1 sequence in mRNA was verified by the amplification of a specific PCR product using primers in the newly identified exon I. Thus, we discovered that the human D1A gene which was previously thought to be intronless has a small intron in its 5' noncoding region.

Transcription initiation site was then determined using S1 nuclease analysis with different primers as well as 5'-RACE during FY 92. Using S1 probes made with primers located upstream of intron 1, diffuse bands were obtained suggesting that transcription is initiated between nucleotides -1061 and -1040. In addition, sequencing of the 5' ends of D1A cDNAs made by the 5'-RACE method revealed that all the clones ended at the adenosine at position -1040. Thus, the findings of both S1 mapping and 5'-RACE indicated that there is no exon upstream of -1061. We concluded that the 5' untranslated region of the human D1A transcript is about 920 nucleotides long, and exon 1 of the D1A gene is about 440 bases. Our assignment of transcription initiation sites, the presence of a small intron and the known 3' extent of the D1A cDNA are all consistent with the previously reported mRNA length of around 4 kb.

Sequence analysis of the region upstream of the transcription initiation site revealed that the promoter region of this gene has neither a TATA box nor a CAAT box. This gene is also highly enriched in G + C content, reaching 80% in some portions. These are all features of "housekeeping genes". In addition, putative sequences for several transcription factor binding sites were seen. The gene has several putative Sp1 binding sites as well as inverted G.C boxes (GGCGGG). Sequences homologous to AP1 binding site, a cAMP response element and multiple possible AP2 sites were identified. The functional significance of these putative transcription factor binding sites in the expression of the D1A gene are currently under investigation. Thus, the human D1A gene belongs to the category of tissue-specific, regulated genes that lack a TATA box.

Studies of the promoter/enhancer activity of the 5' flanking region of the human D1A gene and its cell specificity were begun during FY91 by constructing various 5' deletion mutants (A through C) of the 2.3 kb upstream fragment and subcloning them in pCAT-Basic (Promega) upstream of the translation initiation site of the chloramphenicol acetyltransferase (CAT) gene. Length of the inserts and the 5'-3' orientation relative to the CAT gene were verified by restriction analysis and sequencing. During FY92, transcriptional activity of these fragments were studied in several different cell lines. In NS20Y, which expresses the D1A receptor endogenously, all deletion mutants were transcriptionally active, fragment C (Hind III-Pst I) being most active, compared with pCAT-Basic. Thus, a region of strong enhancer activity located between bases -1341 and -1103 (Hind III-Sac II), and another with silencer activity between -1731 and -1342 (Sma I-Hind III), were identified. In contrast to NS20Y cells, none of the deletion constructs showed substantial transcriptional activity when transfected into C6, HepG2 or NB41A3 cells.

During FY92, more detailed analysis of the critical regulatory elements of the D1A gene were conducted. Eight small 5' deletion mutants of fragment C were prepared and subcloned in pCAT-Basic upstream of the CAT gene and their transcriptional activity tested in three cell lines. In NS20Y cells, the mutant that contained fragment VI (-1198 to -236) was the most active compared with other constructs, while fragment VII showed the most activity in NB41A3 and C6 cells although the latter were considerably lower than the CAT activity obtained in NS20Y cells. The different pattern of transcriptional activity of these deletion mutants between NS20Y and other

cell lines might give a clue about the elements necessary for cell type specific expression of this receptor gene.

In FY92, studies aimed at the interaction of enhancer elements of the human D1A gene with transcription factors were initiated. Both DNase I footprinting and gel mobility shift assays were carried out. A 5' end-labeled, double-stranded DNA probe extending from bases -1200 and -1000 was generated by PCR. In addition to nuclear extract from NS20Y cells, purified human Sp1 and recombinant human AP2 were used in these studies. Several different DNA fragments based on sequences stretching different parts of the probe sequence were generated and used as competitors. Based on sequence homology analysis, the 5' flanking region of the human D1A gene in the segment covered by the probe has two putative Sp1 binding sites at -1149 and -1120. Although NS20Y nuclear extract protected segments -1161 to -1115, -1113 to -1092 and -1076 and -1060 against DNase I, pure Sp1 gave no protection at any site suggesting perhaps these putative Sp1 binding sites are not functional in this gene. Three putative AP2 binding sites are also identified based on sequence homology, at nucleotides -1145 (site A), -1123 (site B), and -1094 (site C). The findings of several experiments suggested that AP2 binds to both sites A and C but the binding to site A has a higher affinity and perhaps is more critical in regulating the expression of the D1A gene. In addition, AP2 in NS20Y cell extract is involved in interacting with the 5' flanking region of the human D1A gene primarily at site A. Factors other than AP2 are also essential in this complex interaction.

The responsiveness of the promoter/enhancer elements of the human D1A gene to various pharmacologic stimuli was also studied in FY92. NS20Y cells were transfected with the four CAT constructs having the D1A 5' deletion mutants A through C and treated with various agents. Because of the presence of putative binding sites for AP1 which mediates the nuclear effects of phorbol esters and AP2 which mediates the effects of cAMP and phorbol esters, drugs that stimulate or augment these systems were used. Preliminary experiments suggested that cpt-cAMP, TPA, forskolin and the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine were all ineffective in modulating CAT expression after 8 hrs of treatment. In addition, DA was used with no significant effect on the transcriptional activity of these constructs. Currently, additional experiments are being conducted to address this issue further.

Regulation of the endogenously expressed D1A receptor is also being investigated in NS20Y cells. These sets of experiments were begun during FY91 and continue during FY92. Both dose-response and time-course experiments are being conducted using a D1A antagonist and the level of the mRNA analyzed by Northern blots and ribonuclease protection assays (RPA). In addition, the effects of cAMP analogs and phorbol esters on D1A gene expression were studied. Parallel experiments looking at D1A receptor binding sites as well as the messages for common transcription factors were conducted. Preliminary experiments revealed no major effects of these treatments on the expression of the D1A gene, however, further quantitative analysis is still being pursued.

2. Promoter analysis of the rat D2 DA receptor gene:

Studies aimed at elucidating the molecular mechanisms involved in the transcriptional regulation of the rat D2 receptor gene were initiated during

the last quarter of FY90. A synthetic oligonucleotide located at the 3' end of the first exon was used as a probe to screen a rat genomic library. Among the positive clones obtained, one (AD2G7) had a 2 kb EcoR I insert that was found to contain sequences from the first exon based on restriction analysis and Southern blot. This EcoRI fragment was subcloned in PUC19 and in pGEM-3Zf(-) giving plasmids pG7E and pG7E2, respectively. A 1.3 kb region of the inserts in pG7E and pG7E2 which was subsequently determined to include the entire first exon, was completely sequenced. Exon 1 sequence is virtually identical to those of rat D₂ receptor cDNAs. We concluded that AD2G7 has part of the rat D₂ receptor gene.

Determination of the 5' end of the D₂ exon I by S1 nuclease analysis was begun in FY91 and completed in FY92. Single-stranded DNA of pG7E2 was used as template in primer extension reaction with an end-labeled oligonucleotide primer D2-P2 followed by EcoRI digestion. The resulting probe was hybridized with rat striatum RNA followed by S1 digestion. The protected fragments were electrophoresed in denaturing gel parallel to DNA sequencing reaction using the same oligonucleotide primer used for preparing the S1 mapping probe. S1 mapping with this 5'-end labeled single-stranded DNA probe and rat striatal RNA showed three strong signals and several weak ones with slower gel mobility. The finding of several rather than a single S1 signal suggested that these nucleotides represent transcription start sites rather than a splice acceptor site. Furthermore, the nucleotide sequence around these signals is generally G + C rich and contains several putative Sp1 binding sites which are features of TATA-less promoters.

The possibility that the 5' ends of exon I are indeed the transcription start sites was further confirmed by sequencing the 5' ends of D₂ cDNAs using rapid amplification of cDNA ends (5'-RACE). The results of the RACE procedure were in general agreement with those of S1 mapping. Among the seven positive clones sequenced, the 5' ends of two were the same adenosine that corresponded to one of the strong S1 mapping signals, and the 5' ends of another five clones matched with one of the weak S1 signals. These results indicated that there is no exon further upstream to exon 1 in the rat D₂ gene, and that this gene has multiple transcription initiation sites located between 321 and 363 nucleotides upstream from the 3' end of the first exon. We have designated the adenosine that corresponds to one of the strong S1 signals and is also one of the 5' cDNA ends generated by RACE as +1. This assignment of transcription initiation sites and the known 3' extent of the rat D₂ cDNA are consistent with the mRNA size of 2.7 - 2.9 kb.

Analysis of the sequence upstream of the transcription start sites indicated that the promoter region of the rat D₂ gene, similar to the human D_{1A} gene, lacks a TATA box and a CAAT box. Like the D_{1A} promoter, the D₂ promoter is also highly rich in G + C content, reaching 80% in some portions, and has multiple putative binding sites for Sp1. However, clear differences are noted between the promoters of the D₂ and D_{1A} genes. While transcription of the D_{1A} gene apparently starts at equal frequency from multiple points, the D₂ gene has a strong preference to begin transcription at the three consecutive nucleotides between -1 and +2 as shown by the major bands protected by S1 nuclease. Furthermore, 8 out of the 17 nucleotides between -6 and +11 of the D₂ gene are identical to the "initiator" sequence, identified in the murine terminal deoxynucleotidyltransferase gene (5'-GCCCTCATTCTGAGAC-3', initiation site is underlined). This initiator-like

sequence in the rat D2 gene may be critical in starting transcription preferentially at the three nucleotides.

Transcriptional activity of the putative rat D2 promoter was also investigated in FY92. To identify the promoter region of the rat D2 gene, five 5' deletion mutants made of overlapping restriction fragments of the 5' flanking region were subcloned in the promoter-less vector pCAT-Basic upstream of the CAT gene in the 5'-3' orientation. In NB41A3 cells, which express binding sites for D2 ligands, strong transcriptional activity was observed with pCATD2-75 but not with the other constructs. These experiments indicated that the D2 promoter is positively modulated by a cis-acting element located between nucleotides -75 and -30 and negatively modulated by elements located between nucleotides -217 and -76 and between nucleotides -852 and -394. S1 nuclease analysis indicated that transcription of the recombinant D2 promoter-CAT gene in this cell line is initiated at the same points as is the D2 gene *in vivo* in the rat striatum. Thus, the authentic D2 promoter drives CAT gene expression. In contrast to NB41A3, none of the CAT constructs showed evidence of transcription in either the C6 glioma cells, the NIH 3T3 embryonic cells or the Hep G2 hepatoblastoma cells, indicating that the D2 promoter is virtually silent in these cells. Thus, the D2 promoter manifests clear cell-type specific pattern of expression.

DNA/protein interaction studies in relation to the rat D2 gene were begun during FY92. The results of the CAT assays suggest that a strong positive modulator is localized between nucleotides -75 and -30 in the D2 promoter. To identify the cis-acting elements and trans-acting factors interacting with this region, we performed DNase I footprinting using nuclear extract from NB41A3 cells and a DNA probe extending from nucleotides -131 to +19 which contains two putative Sp1 binding sites (sites A and B). While purified human Sp1 protein bound to both Sp1 consensus sequences, the nuclear extract showed strong binding to site B which is located between nucleotides -75 and -30 and weak binding to site A. These experiments suggest that Sp1 in the nuclear extract of NB41A3 cells binds to its cognate sequence at nucleotide -48 (site B) but only weakly to the same core sequence at nucleotide -86 (site A). These observations strongly support the possibility that the Sp1 binding site B is crucial for transcription of the D2 gene. Whether the inhibited binding at site A is due to an as yet unidentified factor in the extract partially preventing Sp1 binding, and whether this interference relates to the silencer activity of the -217 to -76 portion of the gene are important issues in the long term objective of this project.

The role of the silencer region located between nucleotides -217 and -75 was further investigated in FY92. Another DNase I-footprinting probe was prepared by PCR extending between nucleotides -225 and -66 encompassing putative Sp1 binding site A. This probe also contains a putative AP2 binding site at base -146. Surprisingly, footprinting with NB41A3 extract showed that site A and a stretch of three TGGG repeats immediately upstream to site A were the only DNase I protected sequences in this region. The AP2 consensus sequence was not protected by NB41A3 extract. To test the ability of this TGGG/Sp1 A sequence in modulating transcription of the D2 gene, an oligonucleotide was synthesized based on this protected sequence and subcloned upstream of the pCAT-D2-75 which is most active transcriptionally. The resulting construct (pCAT-D2-115) was used in a transient expression assay in NB41A3 cells. As expected, CAT activity of pCAT-D2-115 was

significantly lower than that obtained with pCAT-D2-75 (about one-third), indicating that the TGGG/Sp1 A sequence is important in silencing the D₂ gene.

We began addressing the functional significance of the "initiator"-like sequence in the rat D₂ gene during the last quarter of FY92. Plasmid constructs having the enhancer regions (pCAT-D2-75 which included Sp1 site B) with or without the initiator are prepared. These are designated pCAT-D2-75(+12) and pCAT-D2-75(-17) respectively. Their transcriptional activity are now being compared. Footprinting with NB41A3 cells has not thus far shown significant protection at the initiator sequence. The extract used is not enriched in any way and the factors binding to such sequences are expected to be of low abundance.

The potential role of exon 1 in modulating the transcription of the rat D₂ gene was also investigated in FY 92. Exon 1 of this gene has no coding region but is rich in G + C content. To address this issue, two 3' deletion mutants, pCAT-D2-75(+130) and pCAT-D2-75(-17), were created from pCAT-D2-75, which itself contains essentially the entire first exon (down to +278). In NB41A3 cells, both these constructs had significant promoter activity. In HepG2 and NIH 3T3 cells, where pCAT-D2-75 or other pCAT-D2 constructs extending down to +278 are inactive, pCAT-D2-75(+130) gave low but significant promoter activity in both cell lines, while pCAT-D2-75(-17) gave weak promoter activity in HepG2 cells only. These observations may suggest a significant role for exon 1 sequences particularly between +130 and +278 in cell-specific expression of the D₂ gene in NB41A3 but not in HepG2 or NIH 3T3 cells.

Regulation of the expression of the rat D₂ gene by pharmacologic agents and modulators of second messengers were also begun in FY92. Transient transfections of NB41A3 cells with D₂ 5' deletion mutants were done followed by various treatments. No significant effects have thus far been observed either in response to DA, quinpirole or spiperone. Increased transcriptional activity was observed following cotreatment with forskolin and IBMX of pCAT-D2-852 but not pCAT-D2-75. Studies aimed at elucidating the effect of forskolin and IBMX treatment on the endogenous D₂ promoter are currently planned using solution hybridization of RNA from NB41A3 cells with a D₂ riboprobe. In addition, identification of the cis-acting elements responsible for the positive modulatory effects of forskolin/IBMX is planned. Because of the relatively low level of D₂ expression by NB41A3 cells, co-transfection with a D₂ expression vector and the test pCAT plasmids will be performed.

3. Analysis of the 5' flanking region of the rat D₃ receptor gene:

The D₃ receptor is expressed primarily in the limbic parts of the brain such as olfactory tubercle and hypothalamus but also in the nucleus accumbens. The latter may be important in modulating of motor effects of central dopaminergic transmission. Interestingly, many of the DA agonists traditionally thought to be D₂ specific and have motor or "antiparkinsonian" effects have now been found to have higher affinity for the D₃ receptor compared with the D₂ receptor. Thus, many of the behavioral effects of these drugs may well be mediated through the D₃ receptor subtype. Consequently, the Genetic Pharmacology Unit has recently begun investigating the genetic

regulation of this receptor. Studies aimed at characterizing the 5' upstream portion of the rat gene encoding the D₃ receptor were initiated during the last quarter of FY92. Experiments to date have attempted to define the 5' extent of the D₃ message. To accomplish this goal, the 5'-RACE procedure was applied to rat olfactory tubercle RNA using a D₃ specific primer. The RACE products have been cloned in a plasmid vector and screened with a D₃ specific primer. Preliminary results indicate that at least 50% of the clones screened contain D₃ inserts. Several of these positive clones have now been selected randomly and are being analyzed.

4. Expression of peripheral DA receptors:

Although ample pharmacologic evidence exists for the presence of DA receptors in renal tissue, their specific messages had not been previously identified. Low message levels is one possible explanation. To obviate this problem, we used the sensitive and specific mRNA detection method, RPA, with a rat D_{1A} riboprobe and RNA from the rat proximal convoluted tubule. A specific D_{1A} message was detected. A similar strategy was used successfully to detect D_{1B} message in different segments of the nephron. In FY92, the D₂ receptor message was also amplified from various nephron segments using RT/PCR. This set of observations is the first demonstration of three different DA receptor genes expressed in the kidney.

5. Genetic regulation of the rat BDNF gene:

Brain derived neurotrophic factor (BDNF) is a recently identified and cloned protein. The primary interest of the Genetic Pharmacology Unit in BDNF is because of its trophism for not only cholinergic neurons (like NGF) but also for dopaminergic neurons. Although the growth-promoting actions of BDNF have been the subject of much scrutiny, regulation of transcription of this gene has not yet been adequately addressed. Preliminary experiments have shown that this trophic factor does not diffuse readily into brain tissue even when administered into the ventricular spaces. Thus, modulation of expression of the endogenous gene appears to be the only viable means of augmenting BDNF levels in the brain.

The primary objective of this project is to localize and determine the function of key regulatory DNA elements in the 5' flanking region of the BDNF gene to gain insight into means of modulating its expression. This project was initiated during the last quarter of FY92. To define the most upstream exon of this gene, we employed the 5'-RACE procedure using rat cerebellar RNA known to express this gene. To date, we have isolated 18 candidate clones and sequenced some. The sequence of two agree with the published BDNF cDNA sequence up to a point and then both diverge from the published sequence and from each other suggesting the possibility of the presence of splice variants. The latter is currently being investigated and other candidate clones analyzed. Using new sequence information from the most 5' exon, genomic library screening will be carried out.

6. Regulation of POMC gene transcription:

The primary goal of this project is to determine crucial regulatory elements in the 5' flanking region of the POMC gene and to provide information on

their cognate proteins. This gene has been originally used as a model system for investigation by the Unit

In FY89-FY91, novel DNA elements thought to play a role in the transcriptional response of the POMC gene to agents which activate second messenger pathways in clonal corticotrophs (AtT-20), were localized and characterized. We have identified an area between -137 and -106 of the murine POMC gene as a possible target for trans-acting factors. This region contains two elements, separated by 11 bp, which exhibit homology to consensus sequences for AP-1 and AP-2 binding sites, respectively. These elements appear to bind distinct nuclear proteins and exhibit cooperative interaction. Since treatment of corticotrophs with agents that activate phosphatidylinositol or AC pathways leads to increased POMC gene transcription, we have investigated in FY 92 the possible second messenger transducing capabilities of the -137 to -106 segment. We have fused an oligonucleotide made of this sequence upstream of the SV40 promoter in a CAT expression vector yielding pCAT-POMC-137/106. Transient transfection of AtT-20 cells revealed approximately 2-fold increase in CAT activity compared with the enhancer-less vector. Alteration of this construct by point deletion of a single C from the AP2-like sequences resulted in loss of this enhancer activity. No significant change in CAT expression was seen in response to forskolin and/or TPA treatment, suggesting that the -137 to -106 region of the POMC gene is not by itself involved in AP1 or AP2 mediated transcription. Other factors that interact with this sequence to confer its enhancer activity remain to be explored. Interestingly, preliminary experiments suggest that this segment may be a target for transcription activating actions of corticotropin releasing factor (CRF). Thus, CRF effects may not exclusively be due to increased intracellular cAMP.

7. Gene Transfer Project

The ability to control the expression of specific genes in specific cells is an important goal of the Genetic Pharmacology Unit. One technique that has shown some promise in other cellular systems is that of lectin-mediated endocytosis of plasmid DNA. In FY91 and FY92, lectins such as concanavalin A (con A) and wheat germ agglutinin (WGA) were covalently coupled to polylysine using a variety of conjugation techniques. Lectin-polylysine conjugates were then mixed with expression vector DNA (carrying either the bacterial β -galactosidase or CAT genes) and added to various cell lines in culture (AtT-20, NS20Y, PC-12, Swiss 3T3 or NG-108). To date, multiple attempts at obtaining significant expression of the reporter gene in the cells used have been unsuccessful suggesting that the technique may not be readily applicable to these cells. Because of the unsuccessful nature of these experiments, several studies were designed to examine whether the conjugates alone (without DNA) were capable of entering cells in culture. The results indicated that Swiss 3T3 and NS20Y cells consistently internalize ConA and WGA conjugates, respectively. Since the CAT reporter plasmid was expressed in both cell lines when introduced by CaPO_4 -mediated transfection, two possibilities were considered: (1) the reporter plasmid did not enter the cells with the conjugate (i.e., steric hindrance was not overcome by the ionic bonds holding the plasmid to the polylysine); and (2) the reporter plasmid was not expressed in the cells in the presence of the conjugate; i.e., the conjugate may have some effect of its own that hinders transcription or translation of CAT.

To examine the first possibility, the CAT vector was labeled with ^{32}P and the efficiency of ConA-polylysine mediated transfer vs. CaPO_4 -mediated transfection of Swiss 3T3 cells was investigated. Relative to control cells treated with labeled DNA without CaPO_4 , the transfection procedure introduced 5-10-fold more of the labeled plasmid into the cytosolic compartment of the cells. However, ConA-polylysine was completely ineffective. Although confirmatory experiments using higher (probably toxic) concentrations of ConA-polylysine are planned, it is likely that lectin-mediated transfer of DNA will not work in the cell lines investigated so far. It is possible that the previously reported work utilizing an asialoglycoprotein receptor targeting conjugate for transfer of plasmid DNA into hepatocytes is applicable only to hepatocytes or to the specific receptor internalization mechanism employed. Current objectives include assessment of other receptor targeting systems, primarily those involving receptor-mediated endocytosis instead of absorption-mediated internalization seen with lectins. Along these lines, recent progress in DNA transfer using transferrin-polylysine and transferrin-protamine, as well as virus-mediated enhancement of transfer will be pursued. Since the C fragment of the tetanus toxin binds to nerve terminals specifically (of particular interest to us) and since the capacity of the internalization process to accommodate large ligands has not been investigated, we will continue to pursue gene transfer experiments using the C fragment.

8. Protection against neuronal degeneration:

The discovery that MPTP causes death of dopaminergic neurons in humans and results in a clinical picture indistinguishable from idiopathic Parkinson's disease raised several hypotheses about the etiology and pathogenesis of this disorder. In addition, recent attempts to find agents to protect against neural degeneration due to MPTP or other toxins (perhaps some endogenously produced) generated new hope for the treatment of patients with Parkinson's disease. The Genetic Pharmacology Unit has been investigating novel approaches to protect or revert experimentally induced DA neuron toxicity during the last quarter of FY92 in collaboration with the Department of Pharmacology, Creighton University, Omaha, NE. Interestingly, we have found that administration of the adenosine A_1 agonist CHA prevents the depletion of striatal DA induced by MPTP in mice. This was clearly A_1 mediated since it was blocked by an A_1 antagonist but not by an A_2 antagonist. The mechanisms underlying this protective effect will be investigated. We have also used colchicine, which has recently been reported to increase expression of the BDNF gene. Hoping that augmented BDNF levels may provide a neuroprotective effect against MPTP in vivo, as has been demonstrated in vitro, we administered colchicine to mice acutely treated with MPTP. Preliminary experiments so far have shown no consistent effect.

Neurophysiological Pharmacology Section

This project involves investigation of the function of specific neurotransmitters in regulating neuronal activity in the basal ganglia with an emphasis on how the nigrostriatal dopamine system regulates basal ganglia output. In FY92, the Section has continued to study the roles of the different DA receptor subtypes in modulating basal ganglia output, the net

effect of DA receptor stimulation on specific striatal efferents, and the compensatory mechanisms induced by reductions in DA input to the striatum. The role of glutamate in mediating the effects of DA and in regulating tonic activity in the basal ganglia has also been investigated. The long range goal is to devise better therapies for the treatment of neurologic diseases in which the DA system and basal ganglia are implicated, including Parkinson's disease, tardive dyskinesia and Huntington's chorea.

1) D₁ and D₂ DA Receptors in the Basal Ganglia: In Vitro Studies

In previous years we demonstrated that it is necessary for postsynaptic D₁ and D₂ receptors to be simultaneously stimulated for the induction of processes once thought to be independently mediated by the D₂ receptor. In addition, we found that the effects of stimulating the individual DA receptor subtypes in the basal ganglia are altered by chronic DA denervation. These observations have led to a fundamentally new and exciting perception of the relative roles of the D₁ and D₂ receptors located in the basal ganglia, and have had important implications for using DA agonists and antagonists to treat neurologic disease.

In FY92 we have continued to explore these issues through intracellular recording studies using a tissue slice preparation from the striatum of normal rats and from rats whose cells have been lesioned. Studies sought first to determine the effect of D₁ receptor agonists in this preparation and, further, to study the effect of chronic loss of dopaminergic input to striatum on D₁ receptor-mediated responses. Rats were pretreated (6-8 weeks) with a single unilateral injection of 6-hydroxydopamine (6-OHDA) to lesion DA cells.

Application of a D₁ agonist induced an inhibitory effect on striatal neurons with dopaminergic innervation intact: (±)SKF 38393 predominantly decreased neuronal excitability contralateral to the lesion and in sham-operated rats. In contrast, the majority of striatal neurons in tissue ipsilateral to 6-OHDA lesions responded to the D₁ agonist SKF 38393 with an apparent increase in excitability. These results suggested that a change in the function of the striatal D₁ receptor or coupled intracellular processes occurred following chronic dopaminergic denervation. However, studies carried out in FY92 to confirm the role of the D₁ receptor in mediating this excitatory action of (±)-SKF-38393 have produced a different picture. The D₁ agonist fenoldopam and the inactive S(-) enantiomer of SKF 38393 did not mimic the excitatory effects of (±)-SKF-38393 in the lesioned animals. Unlike (±)-SKF-38393, the predominant effect of fenoldopam on neurons ipsilateral to 6-OHDA-induced DA cell lesions was inhibitory. Moreover, application of the inactive enantiomer S(-)-SKF-38393 revealed weak inhibitory and excitatory actions which appeared to mimic that of the racemic compound. Our results indicate, therefore, that (±)SKF-38393-induced increases in the excitability of neurons ipsilateral to DA cell lesions are unlikely to be mediated by the D₁ receptor. In contrast, effects observed with systemic administration of (±) SKF 38393 are clearly stereoselective and specific for the active enantiomer. These results raise significant questions about the interpretation of in vitro or iontophoretic neurophysiologic studies relying on (+)- or (±)-SKF-38393 as a prototypic D₁ agonist.

In addition to investigating the effects of D₁ agonists on striatal tissue from 6-OHDA-treated rats, we have also asked whether membrane properties exhibited by striatal neurons located ipsilateral to 6-OHDA-induced lesions of DA cells differ appreciably from the properties exhibited by neurons in contralateral and control tissue. Patterns of evoked spike activity and the direction/extent of rectification in current-voltage relationship data are not altered by the long-term loss of dopaminergic input to striatum. Moreover, denervation has no appreciable effect on either the resting membrane potential or input resistance exhibited by striatal neurons with respect to either the pattern of evoked firing or current-voltage relationship. However, among neurons exhibiting nominal rectification in current-voltage relations, a time constant describing the early onset of hyperpolarizing membrane transients (τ^*) was significantly smaller for cells located ipsilateral to the lesion compared to contralateral and sham control data. This finding suggests that one or more factors which interact to shape the early onset of membrane transients such as voltage-dependent conductances, synaptic activity, and cable properties may have been modified by denervation. The apparently selective effect of denervation on neurons with nominal rectification could indicate distinct types of neurons with categoric differences in the susceptibility of certain membrane parameters or reflect an ability of voltage-dependent conductances in neurons with marked rectification to mask changes in membrane parameters affecting τ^* .

2. In Vivo Effects of DA Agonists: Globus Pallidus

Insight into the function of dopamine in the basal ganglia has been sought in vivo studies in which the effects of systemically or locally administered dopaminergic agents on firing rates of cells in the substantia nigra pars reticulata and the globus pallidus have been examined. While it is clear that both D₁ and D₂ receptors are involved in mediating the effects of DA on basal ganglia output and on behavior, there is still debate with respect to the actual effect of DA on the activity of the various striatal output neurons in vivo. Previously, we determined that drugs like apomorphine which stimulate both D₁ and D₂ receptor subtypes induce marked increases in the firing rate of what we believed was the predominant cell type in the globus pallidus of locally anesthetized gallamine-immobilized rats. This effect has been believed due to DA-mediated inhibition of striatopallidal neurons. These pallidal cells, referred to as the Type II pallidal cells are spontaneously active, typically firing 10-80 spikes/sec and are identified by their biphasic positive/negative extracellularly recorded action potential. However, recently we have focused on a second population of pallidal cells with slightly different neurophysiologic properties. These cells exhibit a biphasic negative/positive action potential when recorded extracellularly and are termed pallidal Type I cells (because their extracellular action potential is initially negative, as are the Type I striatal cells). In population studies, it was determined that there were no significant differences in the mean firing rate, firing pattern or number of cells/track between the two cell types and they were initially thought to be the same cell type viewed from a different perspective by the extracellular electrode. However, in contrast to the dramatic increase in activity seen with the positive/negative Type II pallidal cells when apomorphine is administered, Type I pallidal cells show mainly a decrease in firing rate with systemically administered apomorphine. Pretreatment with dizocilpine (MK801) changes the pattern of response to apomorphine only with regard to Type II pallidal

neurons. Thus, two distinct globus pallidus cell types have been identified based on their observed extracellular waveform, and they are differently affected by DA receptor stimulation. The discovery that apomorphine exerts an opposite effect on a second cell type in the globus pallidus raises interesting questions about pallidal circuitry. Antidromic stimulation studies indicate that both pallidal cell types project to the subthalamic nucleus and to the substantia nigra. Their conduction velocities differ, however; type I velocity is faster. Further studies on the nature and roles of these two pallidal cell types is planned for the coming year.

3. Effects of DA Depletion on DA Receptor Function: Substantia Nigra Pars Reticulata

The substantia nigra pars reticulata, like the pallidal complex, is a major basal ganglia output nucleus. Both receive inhibitory input from the striatal nucleus and excitatory inputs from the subthalamic and pedunculopontine tegmental nuclei. However, it is clear that different striatal neurons project to the globus pallidus and to the substantia nigra pars reticulata. Models of basal ganglia function currently found in the literature frequently depict DA as excitatory with respect to the striatonigral neurons. This has been inferred from the increases in GABA receptors in the substantia nigra and the decreases in neuropeptide synthesis in striatonigral neurons after 6-OHDA-induced DA cell lesions. However, with neurophysiologic techniques, we see variable changes in the activity of cells in the substantia nigra pars reticulata in normal animals and considerable plasticity with respect to the response of the reticulata cells in DA-depleted rats after DA agonist administration.

Evidence for variability in D₁ mediated effects emerged in a study comparing two strategies for depleting DA: 6-OHDA-induced DA cell lesions and subchronic reserpine treatment. We have previously shown that DA cell lesions resulting from unilateral injection of the neurotoxin 6-OHDA into the medial forebrain bundle causes significant alterations in D₁ and D₂ receptor-mediated neuronal transmission in the basal ganglia. In contrast to the mixed, generally excitatory responses seen in normal rats, in 6-OHDA-lesioned rats, D₁ agonist administration consistently and significantly reduces the firing rates of substantia nigra pars reticulata neurons. D₁/D₂ synergy is observed; D₁-mediated inhibition is markedly enhanced in the lesioned animals by pretreatment with a D₂ agonist.

On the other hand, in reserpine treated rats, D₁ agonist administration leads to increased firing rate of the substantia nigra pars reticulata neurons, an effect exactly opposite to that observed in the 6-OHDA-lesioned rats. The change induced by reserpine treatment is long-lasting, and occurs within 3-8 hrs after a large dose of reserpine. Thus, whatever mechanism is involved in these processes does not require the longer intervals frequently associated with denervation supersensitivity. These results call attention to the fact that short-term reserpine treatment, frequently used to limit DA release in studies of postsynaptic processes, may produce postsynaptic changes by itself.

When the effects of combining the reserpine and 6-OHDA treatments were explored, the D₁-mediated effect on reticulata activity in these rats resembled that seen in the 6-OHDA-lesioned animals; the reserpine treatment

did not "convert" this response to that seen in unlesioned animals given reserpine. This result suggested the possibility that the initial reserpine-induced release of DA might underlie the difference in substantia nigra pars reticulata responses to D₁ receptor stimulation in the two preparations. Alternative hypotheses include the idea that a time-dependent supersensitivity develops in the 6-OHDA-lesioned rats which overrides the effect of reserpine, or that some factors critical to the effect of reserpine are lost when the DA terminals die after 6-OHDA injections. Unlike the substantia nigra pars reticulata, D₁ agonists induce similar changes in neuronal firing rates in both reserpinized and 6-OHDA-lesioned rats in the globus pallidus. Thus, the mechanisms underlying the two opposite responses observed in 6-OHDA and reserpine-treated rats in the substantia nigra pars reticulata are not affecting all striatal output neurons. Furthermore, it would appear that one cannot explain the different responses in the substantia nigra on the basis of different pallidal effects on the pars reticulata neurons in the two preparations. These procedures appear to bring about qualitative changes in the way D₁ agonists affect striatal cells; mechanisms are clearly very relevant to understanding the pathology and pharmacology of Parkinson's disease and will be studied further in the next year.

D₂ agonist pretreatment had a further dramatic impact on the nature of the response to the D₁ agonist in the reserpine-treated rat: the D₂ agonist did nothing significant by itself with respect to substantia nigra pars reticulata neuronal firing rates, but its administration resulted in the pars reticulata neurons responding to D₁ receptor stimulation with a decrease in rate, as seen in the 6-OHDA-lesioned rats, rather than with an increase in rate as normally observed in the reserpine-treated rats. Thus, the net result of stimulating both receptor subtypes in the reserpinized preparation was, in fact, opposite to the result obtained when only D₁ receptors were stimulated. It is certainly unclear how D₂ receptor stimulation might induce such a dramatic "switch" in the effect of D₁ receptor stimulation in the reserpine-treated rats. Studies examining changes in mRNA for D₁ and D₂ receptors in these animals are underway in collaboration with the Genetic Pharmacology Unit.

4 DA Receptor-Mediated Effects on DA Cell Activity

The existence of a striatonigral feedback system through which the activity of DA neurons is regulated by DA receptor stimulation in the striatum was first proposed in 1963. Subsequently, the significance of the "short-loop" feedback system involving the DA autoreceptors on the DA neurons was appreciated. Our previous studies suggested that certain drugs which appeared selective for the DA autoreceptors had such a profile because of the existence of a greater receptor reserve at D₂ DA autoreceptor sites relative to postsynaptic D₂ DA receptor sites. However, the more recent observation of low levels of the D₃ receptor at these sites raises new complications and potential for further therapeutic specificity. We have begun to reevaluate some of the effects of different DA agonists in light of new information about their relative affinities for D₂ vs D₃ receptors. Many of the drugs which are most efficacious at reducing DA cell firing, such as LY 163502 (quinelorane), N-0923 and quiperole, have efficacies relative to apomorphine, more in line with their affinities for D₃ receptors than D₂ receptors. These are also the agents which are most efficacious at reducing

DA cell activity when administered iontophoretically. This has raised concern about the possibility that D₃ receptors may have some significant autoreceptor role. Additional studies are planned.

5. Excitatory Amino Acids and Basal Ganglia Function: NMDA Receptors

The influence of excitatory amino acid (EAA) systems on basal ganglia function has remained an area of interest to the Section in FY92 from both a basic science and a clinical standpoint. The question of whether tonic glutamate input serves as a driving force behind spontaneous activity in various nuclei in the basal ganglia has been investigated by comparing the effects of both AMPA and NMDA receptor blockade on the firing rate of spontaneously active neurons in various basal ganglia nuclei. Increasing doses of the NMDA antagonist MK801 (dizocilpine) had no effect on spontaneous activity in the striatum, globus pallidus, and substantia nigra pars reticulata. Entopeduncular neurons, however, were partially inhibited by MK801 (30% inhibition from baseline at high dose).

In comparison, the AMPA antagonist NBQX produced a dose-related partial inhibition of activity (approximately 25% inhibition at the highest dose) in both the globus pallidus and substantia nigra pars reticulata. Preliminary results suggests that caudate neurons are also inhibited by NBQX. These results indicate that NMDA receptor stimulation plays a role in the tonic activity of entopeduncular neurons, while AMPA receptor stimulation may have a greater influence in the globus pallidus and substantia nigra. The findings thus far suggest that glutamate-mediated neurotransmission contributes to some of the spontaneous activity in the basal ganglia; moreover, this effect is mediated through actions on specific receptor subtypes.

To further explore the role glutamate receptors play in modulating basal ganglia function, in FY92 we have begun to combine extracellular recording techniques with local injections of neurotransmitter agonists and antagonists. This approach has advantages over iontophoretic techniques because the area covered by the drug is larger, reducing the possibility of failing to expose the whole dendritic surface of the neuron being recorded to the agent to be studied. In our initial studies, we have focused on the role of the subthalamic nucleus as a source of putative tonically active glutamatergic projections to the globus pallidus. Local infusion of NMDA or AMPA elicited increases in the firing rate of globus pallidus neurons in a dose-dependent fashion. In contrast, MK801 produced no discernible effect on basal firing rate in the globus pallidus. Local infusion of NBQX into the globus pallidus did reduce the firing rate of pallidal neurons up to 50% of baseline. Infusion of the GABA-A receptor antagonist bicuculline methiodide into the subthalamic nucleus elicited increases in the firing rate of globus pallidus neurons that were reversed by infusion of the non-selective glutamate antagonist kynurenic acid or the more selective NBQX into the globus pallidus. These results indicate that AMPA receptors mediate, at least in part, the basal activity of globus pallidus neurons as well as the increase in neuronal activity resulting from activation of a presumed glutamatergic input from the subthalamic nucleus.

Preliminary reports from other laboratories have suggested that some glutamatergic drugs are beneficial in animal models of Parkinson's disease. However, researchers in this field have not yet arrived at a consensus which

reconciles both the behavioral and the biochemical literature with respect to the effects of these drugs. We have previously shown that MK801 alters the effects of apomorphine on type I caudate neurons and type II globus pallidus neurons in normal rats. Moreover, the excitatory effect on the D₁ agonist on reticulata cells in reserpine-treated rats and the inhibitory effects of the D₁ agonist on reticulata cells in animals with 6-OHDA-induced DA cell lesions are both also blocked by MK801 treatment and by the competitive glutamate antagonist, CPP. These results are interesting in that they suggest that blocking tonic activity at NMDA receptors does not appear to alter the level of spontaneous activity in most basal ganglia output nuclei but tonic activity at NMDA receptors is necessary to mediate a change in basal ganglia activity associated with alterations in DA receptor stimulation. These results suggest that the therapeutic potential of NMDA antagonists for Parkinson's disease is highly questionable in that blockade of NMDA receptors "upstream" from the globus pallidus would interfere with DA agonist-mediated neuronal transmission through the neostriatum. Thus, while the locomotor stimulant effects of MK801, observed in behavioral studies, and effects in the entopeduncular nucleus (medial globus pallidus) may indicate potential for treating Parkinson's disease, reduction of the DA-mediated effects addressed here suggests otherwise.

In contrast, NBQX does not interfere with normal DA agonist-mediated transmission in the globus pallidus, although in the normal rat, the effects of apomorphine in the substantia nigra pars reticulata do appear modulated by AMPA receptor blockade. It remains to be seen how NBQX would affect transmission in the substantia nigra pars reticulata in DA-depleted rats. These studies will be relevant to preliminary reports that NBQX is beneficial in a primate model of Parkinson's disease and support the Branch's clinical interest in this compound. Further research is clearly warranted in this regard. Future experiments are planned to explore the relative role of the NMDA and AMPA receptors in the basal ganglia with the goal of gaining insight into the therapeutic potential of drugs selective for these receptor subtypes.

6. NMDA Antagonists and Anesthesia

These studies have also provided some interesting insights into differences between putative NMDA antagonists. Ketamine is an NMDA antagonist, similar to MK801, and commonly used to induce anesthesia. However, among non-competitive NMDA antagonists, MK801 is typically chosen as a tool for examining the effects of blocking NMDA transmission, whereas ketamine has a long history as a dissociative anesthetic. In FY92, we compared the effects of MK801 and ketamine on a measure of anesthesia (loss of righting reflex) and two measures of basal ganglia DA function: 1) apomorphine-induced stereotypy; and 2) apomorphine-induced excitation of Type II globus pallidus neurons.

The findings demonstrate that DA-mediated changes in behavior and the activity of Type II globus pallidus neurons are comparably affected by MK801 and by i.v. ketamine, presumably via blockade of NMDA receptors. However, MK801 induced anesthesia only at toxic doses, and ketamine-anesthesia is not associated with effects on DA-mediated changes in globus pallidus activity comparable to those caused by MK801. Thus, ketamine and MK801 have significant differences in their pharmacologic effects. Most importantly,

MK801 does not appear to be an effective anesthetic in rats, generalized NMDA receptor blockade does not appear to underlie dissociative anesthesia, and, therefore, the classification of dizocilpine as a dissociative anesthetic cannot be justified on the basis of currently available data. In addition, the ability of ketamine to block the NMDA receptor complex is dependent upon the method of administration of this compound. It would appear that ketamine anesthesia is neither associated with nor results in a diffuse blockade of the NMDA receptor complex.

Clinical Pharmacology Section

Research conducted by the Clinical Pharmacology Section links the results of basic neuroscience studies carried out by other Branch components to the therapeutic needs of the neurologically disordered patient. Clinical and preclinical studies explore pathogenetic mechanisms that may provide a basis for pharmaceutical interventions that will modify basic disease processes. In addition, transmitter pharmacologic approaches are applied to the development of improved symptomatic therapies. The Section's investigative efforts remain centered on Parkinson's disease and related extrapyramidal disorders and to a lesser extent on Alzheimer's disease and related degenerative dementias.

Extrapyramidal Movement Disorders

Both clinical and preclinical research continues to focus on means to improve the pharmacotherapy of Parkinson's disease and especially on the complications attending chronic levodopa administration to those with advanced disease. Our earlier preclinical findings suggested that changes affecting certain neuronal populations within the basal ganglia ultimately compromise all motor responses to levodopa. Whether these neuronal alterations are attributable to natural disease progression or arise, in part, as a consequence of levodopa toxicity has yet to be determined. In an attempt to evaluate the contribution of chronic levodopa therapy as well as other potential risk factors to the appearance of motor complications, a retrospective analysis was recently completed of levodopa-treated parkinsonian patients who received long-term follow-up at the NIH Clinical Center. In the 52 men and 48 women meeting accession criteria, the average delay from symptom onset to levodopa initiation was 2.4 years and the interval from starting levodopa to the emergence of a motor complication averaged 4.1 years. The time between symptom onset and levodopa initiation appeared unrelated to the interval between the introduction of levodopa and the development of complications. This finding supports the view that the appearance of motor complications primarily reflects the degree of DA system degeneration. As a consequence of this loss, striatal systems are modified by exposure to the nonphysiologic fluctuations in DA levels produced by standard oral dosing regimens (vide infra). Since these secondary striatal changes appear to occur relatively rapidly, there does not appear to be a rational basis for delaying levodopa therapy in parkinsonian patients.

Fluctuations in the antiparkinsonian response to levodopa constitute one of the most frequent complications of dopaminomimetic therapy. Previous evaluations of potential sources of these response variations were extended during the past year by an investigation of the possibility that the development of acute tachyphylaxis could be a contributory factor. We assessed the effects of repeated intravenous levodopa injections in patients

with severe motor fluctuations. During a single day, subjects received their optimal levodopa dose each time motor function returned to baseline. We found their peak antiparkinsonian response to be lower by 20% and their peak plasma levodopa levels lower by 35% following the first dose compared with all subsequent doses. Neither peak dyskinesia scores nor the duration of motor response changed significantly with successive levodopa doses. These results suggest that pulsatile levodopa administration does not acutely alter DA receptor responsiveness, and thus that other pharmacokinetic and pharmacodynamic factors contribute to the dose-to-dose variability that occurs in response to levodopa.

It is generally agreed that continuous dopaminomimetic therapy, by allowing levels of the pharmacologic agent to remain constant at target sites, promptly ameliorates wearing-off fluctuations. We have also previously reported that round-the-clock infusion of the DA precursor for several days not only prolongs the duration of the drug's antiparkinsonian action but also widens its therapeutic window. These response modifications, as a consequence of changing the levodopa treatment schedule from intermittent to continuous, could have important implications for the management of parkinsonian patients. Unfortunately, the impracticality of administering levodopa parenterally for more than a few weeks, necessitates the use of other dopaminomimetics to determine whether longer periods of continuous therapy will confer additional benefit. Currently ongoing studies are attempting to address this problem through the administration of a very long half-life DA agonist and the use of a catechol-O-methyltransferase inhibitor as an adjuvant to levodopa. Related investigations completed during the past year evaluated the continuous dopaminomimetic strategy for the relief of motor complications as well as the contribution of pre- and post-synaptic striatal mechanisms to the pathogenesis of these complications through the continuous administration of the dopamine agonist, lisuride. Following a 3-month round-the-clock infusion of lisuride, the duration of antiparkinsonian action of levodopa increased by 90%, and the therapeutic window for the acutely administered dopamine precursor widened by more than 300%. These benefits were more than 3 times greater than those we previously observed after 9 days of continuous levodopa administration. In contrast to the effects on levodopa pharmacodynamics, the continuous infusion of lisuride did not prolong its action, suggesting a lisuride effect on presynaptic as well as postsynaptic dopaminergic mechanisms. These results lend further support to the view that continuous dopamine replacement ameliorates the motor fluctuations and peak-dose dyskinesias complicating standard levodopa regimens. Our findings further suggest that alterations at both pre-synaptic and postsynaptic levels contributing to these motor complications tend to normalize with the more physiologic stimulation afforded by continuous replacement strategies, especially when given chronically.

The hypothesis that in advanced parkinsonian patients, where the therapeutic index to levodopa has narrowed, partial DA receptor agonists alone or in combination with levodopa may confer clinical benefit has been further explored in two controlled studies completed during the past year. In the first, we found that terguride produced a dose-dependent decrease in levodopa induced dyskinesias (up to 53%) without a concomitant worsening of parkinsonism, but had no significant antiparkinsonian effect when administered alone. In the second, EMD 49980 monotherapy resulted in a mild improvement in parkinsonian symptoms, but when coadministered with levodopa,

had no significant effect on dyskinesias or on the antiparkinsonian effect of the DA precursor. Taken together our results lend additional support to the view that partial DA agonists hold promise in the therapy of patients with advanced Parkinson's disease and provide new insight into the pharmacologic profile of agents that might prove most useful in this application.

Although our animal model studies point to the potential importance of targeting dopaminomimetic therapies in parkinsonian patients to selected receptor subtypes within the D₁ and D₂ families, clinical trials of this hypothesis have been relatively uninformative due to the lack of safe and appropriate pharmacologic agents. Recently, however, we have begun clinical studies with the most highly selective agonist for the D-3 receptor yet identified. A related preclinical study just completed addressing interactions between D₁ and D₂ receptor mediated mechanisms evaluated the effects of G_i protein modification produced by intrastriatal pertussis toxin injection on DA-mediated motor behavior. In rats pretreated with pertussis toxin unilaterally, the administration of the selective D₂ agonist quinpirole induced ipsilateral rotation but the selective D₁ agonist SKF38393 did not. SKF38393 was, however, able to increase rotation induced by quinpirole. The selective D₂ antagonist raclopride and the selective D₁ antagonist SCH 23390 both blocked the effects of quinpirole. SKF38393, but not quinpirole, increased striatal cAMP levels, but quinpirole had no effect; when administered together, quinpirole attenuated the SKF38393-induced increase in cAMP. The quinpirole-induced attenuation of the SKF38393 effect was greater in the intact striatum: in pertussis toxin-injected striatum, quinpirole only attenuated the SKF38393-induced cAMP increase to control levels. This imbalance between intact and injected striatum presumably contributes to the rotation in pertussis toxin-injected rats and emphasizes the crucial role for G proteins in DA receptor interactions.

Previous Section findings that levodopa administration to hemiparkinsonian rats profoundly alters neuropeptides in striatal dopaminoceptive systems were extended during the past year by an investigation of the effects of selective D₁ and D₂ DA receptor agonist stimulation. Rats with a unilateral 6-OHDA lesion of the nigrostriatal pathway were treated intraperitoneally for 7 days with the SKF 38393 or quinpirole by either continuous infusion and intermittent (once daily) injection. Alterations in neuropeptide levels were observed primarily on the denervated side. In comparison to values from lesioned, vehicle-treated controls, intermittent administration of SKF38393 reduced somatostatin (ST) and neuropeptide (NPY), increased neurotensin and dynorphin, but had no effect on enkephalin; continuous SKF38393 decreased NPY, but did not alter levels of the other peptides. Continuous quinpirole elevated ST and NPY levels, but reduced the lesion-induced increases in both neurotensin and enkephalin. Conversely, intermittent quinpirole decreased ST and NPY, increased neurotensin, and had no effect on enkephalin. Dynorphin levels were not altered by either continuous or intermittent quinpirole. These observations suggest that ST, NPY, and neurotensin levels are modulated by both D₁ and D₂ DA receptors: ST and NPY show parallel changes, but alterations in neurotensin are opposite in direction to those of ST and NPY. Striatal dynorphin is influenced only by D₁ receptor stimulation, while enkephalin is modulated only by D₂ receptors. The effects of DA receptor stimulation on downstream striatal peptidergic systems are markedly enhanced by dopaminergic denervation and differ substantially depending on whether the stimulation is continuously or intermittently administered.

The foregoing preclinical results emphasize the need to develop means to study peptidergic function in the brain of living individuals. As an approach to evaluating striatal peptides in parkinsonian patients, we measured the proenkephalin derivative Met5 enkephalin-Arg6-Gly7-Leu8 (MERGL) as well as dynorphin A(1-8) in lumbar spinal fluid under basal and drug-treated conditions. MERGL was significantly reduced in these patients in the basal state compared with age-matched controls and did not change with a steady-state optimal-dose infusion of levodopa. MERGL levels increased with advancing age among normal individuals but not among those with Parkinson's disease. In contrast, dynorphin A(1-8) levels did not differ between patient and control groups, remained unchanged with levodopa therapy, and failed to correlate with age or indices of disease progression. These observations, consistent with postmortem studies on parkinsonian brains but contrary to our findings in an animal model of parkinsonism, suggests that the enkephalinergic abnormality in Parkinson's disease reflects direct involvement of this system by the neurodegenerative process. Since an enkephalin deficiency could contribute to some levodopa-resistant symptoms as well as to certain of the motor response complications found in advanced parkinsonian patients, pharmacologic manipulation of this system may have therapeutic value. Whether the absence of spinal fluid dynorphin changes reflects the striatal situation or the noninformativeness of lumbar CSF samples of this neuropeptide remains to be determined.

Elucidating the manner in which the glutamate system influences dopaminergic effects on motor function may be important to the treatment of movement disorders such as Parkinson's disease. The 2-deoxyglucose autoradiographic technique was used to examine the effect of NMDA receptor blockade on regional cerebral metabolic responses to D₁ and D₂ DA receptor stimulation in rats with a unilateral 6-OHDA lesion of the nigrostriatal pathway. SKF 38393 increased glucose utilization markedly in entopeduncular nucleus and substantia nigra pars reticulata ipsilateral to the lesion, while quinpirole had no effect in these striatal output regions. SKF38393 and quinpirole reduced 2-deoxyglucose uptake to a similar extent in the lateral habenula, a region which receives afferent input from entopeduncular nucleus; quinpirole also decreased glucose utilization bilaterally in nucleus accumbens. Pretreatment with MK801, which had little effect on cerebral metabolism by itself, reduced the effect of SKF38393 in the entopeduncular nucleus and substantia nigra pars reticulata and prevented the effect of quinpirole in nucleus accumbens. MK801 did not alter the SKF38393-induced reduction in glucose utilization in the lateral habenula, but did reduce the effect of quinpirole in this structure. When these drugs were administered in the same manner to a separate group of lesioned animals, MK801 did not affect rotational behavior elicited by SKF38393, but completely eliminated contralateral rotation in response to quinpirole. These findings indicate that D₁ and D₂ receptor-associated mechanisms are differentially influenced by NMDA receptor stimulation. D₂ mediated behavioral and metabolic responses appear to require concurrent NMDA receptor stimulation. On the other hand, the preservation of D₁-mediated rotational behavior and reduced lateral habenula glucose metabolism in the presence of MK801 despite attenuation of the effects of the D₁ agonist in the entopeduncular nucleus and substantia nigra pars reticulata suggests that D₁ receptor-regulated neuronal pathways exhibit varying degrees of sensitivity to NMDA receptor blockade.

Since D₂ receptor-mediated striatal output via the striatopallidal pathway involves glutamatergic projections from subthalamic nucleus to internal pallidum (entopeduncular nucleus) and substantia nigra pars reticulata, blockade of pallidal NMDA receptors may explain the inhibitory effect of MK801 on D₂ agonist-induced motor and metabolic responses. To evaluate this possibility, we have just completed a study of the differential effects of unilateral subthalamic nucleus ablation on rotational responses to D₁ and D₂ agonists in rats with a unilateral 6-OHDA lesion of the nigrostriatal pathway. Four to five weeks after lesioning, D₂ agonist-induced rotation was reduced in subthalamic nucleus-lesioned rats relative to sham controls, although no such reduction in D₁ agonist-induced circling occurred. However, one to two weeks following subthalamic nucleus lesion marked reductions occurred in both D₁ and D₂ agonist-induced rotation in subthalamic nucleus-lesioned rats compared to sham controls. These results lend further support to the view that the subthalamic nucleus contributes primarily to the expression of D₂ mediated motor behaviors, although ablation of the subthalamic nucleus may induce certain time-dependent compensatory mechanisms in other basal ganglia structures which affect D₁ mediated actions.

Two clinical studies completed during the past year assessed the ability of glutamatergic mechanisms to influence extrapyramidal motor function. In the first, motoric effects of central glutamatergic stimulation by the putative glycine prodrug, milacemide, were studied in patients with Parkinson's disease under controlled conditions. When administered alone, milacemide transiently increased overall parkinsonian severity, with rigidity being most profoundly affected. Milacemide did not, however, alter levodopa-induced dyskinesias. These results suggest that drugs acting on the glutamate system can influence motor function in patients with extrapyramidal movement disorders. Specifically, pharmaceutical agents that selectively block certain glutamate receptors may reduce parkinsonian signs, while those that stimulate central glutamatergic function may act to ameliorate hyperkinetic disorders. To evaluate this hypothesis further as well as to explore the clinical implications of findings in the experimental animal suggesting that hypofunction of the glutamatergic subthalamopallidal tract contributes to the hyperkinesia in Huntington's disease, in the second study we evaluated milacemide in Huntingtonian patients using a double-blind placebo-controlled design. Oral doses at maximum allowable levels failed to alter either chorea or cognitive dysfunction. Specific modulatory effects of glycine on the NMDA subtype of glutamate receptors, rather than the AMPA receptors which may predominate on target neurons in the pallidum, could contribute to the therapeutic failure of milacemide in this disorder. A clinical trial of a centrally acting NMDA antagonist will now evaluate differences in the role of glutamatergic mechanisms in the regulation of motor function between parkinsonian and Huntingtonian patients as well as between Parkinson's disease and its rodent model.

Several investigations completed during the past year addressed basic pathogenetic issues related to Parkinson's disease as well as strategies for the development of a successful neuroprotective intervention. In the first series, we evaluated reports suggesting that reductions in mitochondrial respiratory function occur in substantia nigra, platelets, and muscle from parkinsonian patients. To confirm and further characterize the presence of a generally distributed mitochondrial defect, platelets and muscle samples were obtained from the same subjects with Parkinson's disease as well as from

normal controls. Oxygen consumption rates in permeabilized platelets and muscle mitochondria represented by Complex I, Complexes II/III, or Complex IV did not differ between the two groups. Similarly, activities of rotenone-sensitive NADH cytochrome c reductase, succinate cytochrome c reductase, or cytochrome oxidase in platelet or muscle mitochondria were not significantly different between patients and controls. Neither chronic therapy with levodopa/carbidopa alone nor in combination with deprenyl affected any measure of mitochondrial respiratory function. There was no discernable relation between patient age or disease severity and any parameter of mitochondrial respiration. Finally, blood lactate levels following glucose loading were no different in patient and control groups. These results fail to support the occurrence of a generalized defect in any mitochondrial respiratory function in Parkinson's disease, but do not exclude an abnormality in respiratory function confined to substantia nigra. If previously published results from brain tissues are correct, however, the defect would be most compatible with a secondary glial response rather than a primary neuronal deficiency.

A related study attempted to elucidate pharmacologic mechanisms responsible for the reported ability of deprenyl to delay the need for levodopa by parkinsonian patients. Measures of neurologic function as well as indices of free radical scavenging activity were assessed in individuals with Parkinson's disease using a single-blind placebo-controlled crossover design. After one month, deprenyl had decreased the optimal levodopa requirement by 24% (oral) and 16% (intravenous). Levodopa-induced dyskinesias were prolonged by 430%, and antiparkinsonian activity by 44%. Mood improved significantly. One month after withdrawing deprenyl, effects on dyskinesias and mood had yet to return to baseline, suggesting the washout period used in previous studies was too short. There was no change in activities of circulating glutathione peroxidase, glutathione reductase, glutathione transferase, superoxide dismutase, and catalase, nor in levels of lipid peroxide or vitamin E. Deprenyl also failed to modify CSF levels of total glutathione and activities of glutathione peroxidase or superoxide dismutase. These results lend no support to the possibility that deprenyl acts in humans to influence free radical scavenging. Deprenyl effects on levodopa pharmacodynamics and mood complicate the interpretation of previously published investigations of the drug's neuroprotective action and could increase the risk of adverse effects of levodopa.

Clinical studies were also completed during the past year in patients with progressive supranuclear palsy (PSP) and spasmodic torticollis. The first, exploring the contribution of cholinergic dysfunction to the pathophysiology of PSP, revealed a 31% reduction in acetylcholinesterase (AChE) activity in lumbar CSF relative to control levels. Oral administration of the AChE inhibitor, physostigmine (up to 2.0 mg every two hours, six times a day for 10 days), under controlled conditions to these individuals had no effect on neurologic function or on CSF AChE activity. Our results suggest that the doses of physostigmine used were insufficient to produce pharmacologically adequate AChE inhibition within the CNS and thus the need to use safer and more effective cholinomimetics to evaluate the role of cholinergic dysfunction in PSP. In a second series of studies, the effects of calcium channel inhibition (verapamil) and serotonin-1A receptor antagonism (buspirone), were evaluated in double-blind placebo-controlled crossover studies. Verapamil was given in rising doses for 3 weeks patients; buspirone

was administered in rising doses for 4 weeks. Neither drug significantly benefitted symptoms in the group as a whole or in any individual patient.

Functional cerebral imaging especially with positron emission tomographic (PET) scanning and [^{18}F] 2-fluoro-2-deoxyglucose (FDG) can assist in the localization of regional neuronal dysfunction in brain disorders lacking neuropathologic alterations. A comparison of patients with Tourette syndrome against matched controls completed during the past year revealed substantial cerebral differences that distinguish patient and control groups. Specifically, Tourette patients are characterized by decreased metabolic rates in ventral brain, particularly in the orbitofrontal and inferior insular cortices, striatum, and mesial temporal regions. There are concomitant increases in normalized metabolic rates in the superior cortical convexities, including premotor regions and rolandic cortices as well as post-rolandic sensory association areas including the superior parietal lobule. An analysis of correlations between local rates of glucose metabolism disclosed markedly altered relationships between these limbic and motor regions, most strikingly a reversal of functional relationships between the ventral striatum and sensorimotor cortices. These abnormalities appear specific for Tourette syndrome, could be of pathogenetic significance, and will help direct future biochemical probes and clinical trials in patients with this disorder.

Patients with predominantly unilateral parkinsonian signs may provide a unique opportunity to evaluate the cerebral representation of cognitive functions characteristically affected in Parkinson's disease. Twenty hemiparkinsonian patients (ten left and ten right) and ten healthy controls, matched for age and education, were studied with neuropsychologic tests and PET-FDG scans. Both right and left hemiparkinsonians evidenced impaired visuospatial and verbal episodic memory, but had no deficits in executive abilities compared to controls. None of the neuropsychologic test scores distinguished right from left hemiparkinsonians. Glucose metabolic profiles were identical for the three groups in all cortical areas assessed. In the subcortex, only the lenticular hypermetabolism contralateral to the predominant side of motor involvement that characteristically occurs in Parkinson's disease was evident. Correlational analysis revealed that higher glucose metabolic rates in the basal ganglia of hemiparkinsonians were associated with lower visuospatial test scores. In frontal and parietal cortex, decreasing glucose metabolism was positively associated with neurobehavioral function; in temporal cortex, measures of attention and memory decreased with increasing glucose metabolic rates. The relative lack of lateralization in neuropsychological performance and glucose metabolism in cortex suggests that side to side differences in hemiparkinsonian patients tend not to involve higher cortical function.

Dementing Disorders

Previous clinical trials of physostigmine have failed to demonstrate clinically significant antidementia efficacy in patients with Alzheimer's disease. An assessment of the pharmacokinetic characteristics of this cholinesterase inhibitor showed poor oral bioavailability, high plasma clearance, and a short half-life. To overcome these limitations, we evaluated the cognitive effects of physostigmine given by intravenous infusions lasting 6-8 hrs at doses up to maximum tolerated amounts. At these rates, averaging

0.45 mg/hr, AChE inhibition averaged 21% and ranged up to 46%. Neuropsychologic testing disclosed no overall cognitive benefit, although there was a tendency towards improvement in long-term retrieval on the selective reminding test which correlated with the degree of central cholinesterase inhibition. PET-FDG scans revealed significant changes in overall cerebral metabolism, especially in thalamus; alterations in parietal cortex, amygdala and hippocampus correlated with improvement on the selective reminding test. Our results indicate that physostigmine, even at stably-maintained maximum-tolerated dose levels, provides no overall symptomatic relief to those with Alzheimer's dementia, but do not exclude the possibility that adequate cholinergic augmentation by appropriate pharmacologic means might prove beneficial.

Several studies completed during the past year applied the PET-FDG method at both laboratory and clinical levels to the development of more effective therapeutic alternatives for Alzheimer's disease. First, in order to facilitate a comparison of drug effects on regional brain metabolism in rats and humans, an atlas of cerebral regions of interest common to both species was developed. Using this atlas we found that metabolic values and their coefficients of variation for rat and human brain regions considered anatomically similar were significantly positively correlated. The similar distribution of metabolic activity and variations in this activity supports the view that rat and human brain may be phylogenetically linked not only structurally but also functionally. We then compared the effects of various cholinergic agonists and antagonists on regional glucose metabolism in rodents. Scopalamine depressed metabolism in an area of cerebral cortex focused around the parietal region. Rats treated with direct cholinergic agonists (U-80816B, RS86) as well as with an indirect agonist (physostigmine) all showed decreases in cortical energy metabolism similar to scopalamine. When these drugs were given in conjunction with scopalamine, metabolism tended to change in the opposite direction from values obtained by the drug alone. These results suggest that there are complex interactions between pre- and post-synaptic muscarinic receptors and that some of the effects of physostigmine appear mediated by nicotinic receptors.

Applying the same approach clinically, we also examined the hypothesis that degeneration of acetylcholine-containing projections from basal forebrain to cerebral cortex accounts for the dementia of Alzheimer's disease and that restoration of cerebral cholinergic function will improve cognition. Whether the failure of clinical trials of various cholinergic replacement strategies to produce consistent, clinically significant benefit reflects a faulty cholinergic hypothesis or inadequate central drug activity remains unclear. Our PET study showed that amnestic doses of the anticholinergic, scopalamine, given normal elderly volunteers stimulates neuronal function in all cortical regions as evidenced by changes in regional FDG metabolism. This cortical response contrasts with the mainly parietotemporal decrease in metabolism found in untreated Alzheimer patients. Moreover, administration of maximum tolerated doses of the cholinomimetic, physostigmine, rather than tending to normalize cerebral abnormalities in these patients produces a generalized reduction in cortical metabolism. These results suggest that cholinergic denervation alone cannot explain the pattern of cortical dysfunction found in Alzheimer's disease and that cholinomimetic monotherapy may not be able to confer substantial cognitive benefit to Alzheimer patients.

ST levels are also reduced in the brains of patients with Alzheimer's disease. To test whether pharmacologic restoration of this transmitter deficit confers therapeutic benefit, its synthetic octapeptide analogue, octreotide, was administered intravenously at multiple dose levels to Alzheimer patients under double-blind, placebo-controlled conditions in a study just completed. Only about 0.3% of the circulating drug entered the CNS, although at the highest dose levels, spinal fluid concentrations approximated those found in the brains of experimental animals receiving behaviorally effective amounts of octreotide. Neuropsychologic testing, however, showed no clinically significant improvement following injections of up to 30 mg or steady-state infusions at up to 15 mg/hr. Co-infusion of octreotide and physostigmine to one patient also failed to improve intellectual function. PET-FDG scans revealed a generalized decrease in glucose metabolism at maximum octreotide dose levels. Our findings suggest that neural elements normally responsive to ST, including its receptors and other interacting neural networks, are at least partly preserved in Alzheimer's disease. Nevertheless, the inability of this potent and relatively long-lasting ST analogue to improve cognitive performance, despite attainment of purportedly adequate central drug levels and the induction of cerebral metabolic changes, suggests that stimulation of this transmitter system alone will have limited value in the symptomatic treatment of Alzheimer's dementia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02263-16 ET
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical and Pharmacological Studies of Dopamine Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: David R. Sibley, Ph.D. Chief, Molecular Neuropharmacology Section ETB/NINDS Others: Frederick J. Monsma, Jr., Ph.D., Senior Staff Fellow; Elzbieta M. Smyk-Randall, Ph.D., Senior Staff Fellow, Yong Shen, Ph.D., Visiting Fellow, Li-Juan Zhang, Ph.D., Visiting Fellow, Brian N. Atkinson, Ph.D., IRTA Fellow, Jean E. Lachowicz, Ph.D. IRTA/PRAT Fellow, Sara Fuchs, Ph.D. Guest Researcher, and Julie L. Pickholtz, Special Volunteer, ETB/NINDS		
COOPERATING UNITS (if any) Lab Cell Biology, NIMH; Chicago Med. Sch; Molecular & Behavioral Neurosc Ctr. Rutgers Univ.; Ctr. Cell Biology, Sinai Hosp of Detroit; Psych. Dept, Seattle VA Med Ctr; Pediatrics Dept, George. Univ.		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Molecular Neuropharmacology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 7.0	PROFESSIONAL: 6.75	OTHER: 0.25
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews <input checked="" type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The long-term goal of this project is the characterization of neurotransmitter receptor-mediated information transduction, and its regulation, across neuronal membranes. The primary, but not exclusive, model systems under investigation are those for dopamine (DA) receptors. In order to characterize DA and related receptors at the biochemical and molecular levels and study their regulation, there are two major interrelated lines of research which are ongoing: 1) investigation of the cell biology, function and regulation of the receptors at the protein level; and 2) the molecular cloning of the receptor genes and investigation of gene structure and regulation in normal and pathophysiological states. 1. <u>Cell Biology and Regulation of DA Receptors.</u> Characterization of the functional and regulatory properties of D ₁ and D ₂ DA receptors on various neuroblastoma and cDNA-transfected cell lines were continued. The D ₁ receptors were shown to undergo an agonist-induced form of desensitization which is partially cAMP-mediated and involves both functional uncoupling and down-regulation of the receptors. D ₂ receptors were also shown to undergo desensitization in response to agonist treatment but, in contrast to D ₁ receptors, demonstrate an up-regulation response. A variety of anti-peptide antibody probes for DA receptors were developed and used to map the expression of the D ₁ , D ₂ and D ₃ receptor proteins at the cellular level in the CNS. 2. <u>Molecular Cloning of DA and Other Receptors.</u> A cDNA encoding a second D ₁ receptor (designated D _{1B}) linked to adenyl cyclase in rat kidney was identified and cloned. The distribution of the D _{1A} and D _{1B} receptors were mapped in the kidney. Work continued on the cloning of a third "D ₁ like" receptor which apparently is linked to the stimulation of phosphatidylinositol turnover and calcium mobilization. The genes for the D _{1A} , D _{1B} , and D ₂ receptors were isolated. Transgenic "knock-out" experiments for several of the dopamine receptor subtypes were initiated. Two completely novel serotonin receptors were cloned and expressed. Several other cDNA clones encoding putative "orphan" G protein-linked neurotransmitter receptors were identified.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02826-02, ET
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Regulation of Transmitter Receptor Genes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. Maral Mouradian, M.D.	Head, Genetic Pharmacology ETB/NINDS
Others:	Takashi Minowa, Ph.D.	Visiting Fellow ETB/NINDS
COOPERATING UNITS (if any) Dept. Physiology, Uniformed Services Univ. of the Health Sciences; Sect. Pediatric Nephrology, Georgetown Univ Med Ctr; Dept. Pharmacology, Univ. Nebraska, Omaha, NE.		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Genetic Pharmacology Unit		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	3	PROFESSIONAL: 2 OTHER: 1
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>In FY 92, the Genetic Pharmacology Unit focused on the transcriptional regulation of genes encoding <u>dopamine receptors</u>, BDNF and POMC:</p> <ol style="list-style-type: none"> <u>Transcriptional regulation of the D_{1A} dopamine receptor gene.</u> A human genomic clone having the 5' flanking region of the D_{1A} gene was isolated and sequenced during FY 91. In FY 92, this gene was found to have a small intron in its 5' untranslated region; the true exon 1 was identified and transcription found to start at multiple points. The transcriptional activity of several <u>deletion mutants</u> of the D_{1A} promoter region was determined by subcloning them in a CAT expression vector. Strong enhancer and silencer regions were identified. Footprinting and gel shift assays suggested that AP2 in NS20Y nuclear extract binds to an AP2 consensus sequence in the promoter of this gene. No Sp1 binding was seen although the D_{1A} promoter has multiple consensus sequences for Sp1. We concluded that the D_{1A} gene, like "housekeeping" genes, lacks a TATA box but is tissue-specific and highly regulated. <u>Promoter analysis of the D₂ dopamine receptor gene.</u> The rat genomic clone encoding the D₂ receptor, which was isolated in FY 91, was further characterized in FY 92. Although transcription starts at multiple points, a clear preference is noted for three consecutive nucleotides located in an "initiator"-like sequence. Promoter analysis revealed an enhancer region harboring a functional Sp1 binding site and a silencer region having an Sp1 consensus sequence where Sp1 in NB41A3 nuclear extract binds only very weakly. In addition, sequences in exon 1 are found to contribute to the transcriptional regulation of this gene and to its cell-specific expression. We concluded that the D₂ gene lacks a TATA box but appears to have a functional "initiator" element. <u>Analysis of the 5' flanking region of the D₃ receptor gene.</u> This project was initiated during the last quarter of FY92. A D₃ specific cDNA has been amplified from rat olfactory tubercle and several candidate clones harboring its 5' extent isolated. <u>Genetic regulation of the rat BDNF gene.</u> Experiments to clone the gene for brain derived neurotrophic factor were begun during the last quarter of FY 92. To date, 18 candidate clones harboring the most upstream portion of this transcript have been isolated and are being sequenced. <u>Regulation of POMC gene transcription.</u> The previously identified -137 to -106 region of this promoter was found to have significant enhancer activity when fused upstream of the SV40 promoter. 		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02139-18 ET
PERIOD COVERED October 1, 1991 through September 31, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pharmacology and Physiology of the Substantia Nigra and Basal Ganglia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Judith R. Walters Chief, Neurophysiological Pharmacology Section ETB/ NINDS	
Others:	Debra Bergstrom Pharmacologist ETB/NINDS	
	Michael Twery Senior Staff Fellow ETB/NINDS	
	Kai-Xing Huang Special Volunteer ETB/NINDS	
	Mark Kelland Senior Staff Fellow ETB/NINDS	
	Lisa Thompson Staff Fellow ETB/NINDS	
	Robert Soltis PRAT Fellow ETB/NINDS	
COOPERATING UNITS (if any) Clinical Pharmacology Section, Experimental Therapeutics Branch		
LAB/BRANCH Experimental Therapeutics Branch, CNP		
SECTION Neurophysiological Pharmacology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	6.9	PROFESSIONAL: 5.9 OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>1) <u>D1 and D2 Dopamine Receptors in Basal Ganglia: In Vitro Studies.</u> Studies in striatal slices utilizing intracellular recording techniques find patterns of evoked spike activity and the direction/extent of rectification in current-voltage relationship data are not altered in striatal neurons following 6- week (DA) cell lesion. However, among neurons exhibiting nominal rectification in current-a-voltage relations, a time constant describing the early onset of hyperpolarizing membrane transients was significantly smaller than in control. SKF38393 effects on neuronal excitability are altered after DA cell lesion but this drug's action is unrelated to D1 receptors, raising questions about in vitro use of SKF 38393 as prototypic D1 agonist.</p> <p>2) <u>In Vivo Effects of Dopamine Agonists: Globus Pallidus.</u> Two distinct globus pallidus cell types have been identified based on their extracellular waveforms and response to DA receptor stimulation. The cells' opposite responses to systemic apomorphine raises questions about basal ganglia circuitry.</p> <p>3) <u>Consequences of Dopamine Depletion in the Basal Ganglia.</u> Evidence for variability in D1- mediated effects observed: after reserpine treatment, D1 agonist administration leads to an increase in the firing rate of the substantia nigra pars reticulata neurons, an effect exactly opposite to that observed in the 6-OHDA lesioned rats. Moreover, the net result of stimulating both receptor subtypes in the reserpinized preparation was, in fact, opposite to the result obtained when only D1 receptors were stimulated.</p> <p>3) <u>Role of Excitatory Amino Acid Receptor Subtypes, AMPA, and NMDA in Basal Ganglia Function.</u> The NMDA antagonist dizocilpine had no effect on spontaneous activity in the striatum, globus pallidus, and substantia nigra pars reticulata. although entopeduncular neurons were partially inhibited by dizocilpine(MK 801). In contrast, the AMPA antagonist NBQX produced a dose-related partial inhibition of activity in the globus pallidus, substantia nigra pars reticulata, and caudate neurons when given systemically. Local infusion of NBQX reduced pallidal activity and partially blocked effects of activating the subthalamic nuclei. Thus, tonic glutamate input serves as a driving force behind some spontaneous activity in various nuclei in the basal ganglia. Studies with glutamate antagonists ketamine and MK801 have indicated that MK801 does not appear to be an effective anesthetic; ketamine anesthesia is neither associated with nor results in a diffuse blockade of the NMDA receptor complex.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02265-16 ET
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pharmacology, Biochemistry and Physiology of Central Neurotransmitters		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Thomas N. Chase, M.D. Chief ETB/NINDS Others: Jeff Anderson, PhD, IRTA Fellow; Robert Boldry, PhD, IRTA Fellow; Daniele Bravi, M.D. Special Volunteer; Thomas M. Engber, PhD, Senior Staff Fellow; Stella Papa, M.D., Visiting Fellow; Christopher Randolph, PhD, Senior Staff Fellow; John Roberts, M.D., Clinical Associate; Young Sohn, M.D., Special Volunteer.		
COOPERATING UNITS (if any) Georgetown Univ, Wash, D.C.; Upjohn Co, MI; Hosp De La Salpetriere, Paris; NIMH; NIDCD; NIA; NIDR; Merck Sharp & Dohme; Univ Pavia, Italy; Royal Ottawa Hosp.; Canada		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Clinical Pharmacology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: <div style="display: flex; justify-content: space-between; width: 100%;"> 10.0 </div>	PROFESSIONAL: <div style="display: flex; justify-content: space-between; width: 100%;"> 8.0 </div>	OTHER: <div style="display: flex; justify-content: space-between; width: 100%;"> 2.0 </div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <ol style="list-style-type: none"> 1. The interval from the onset of symptoms to the introduction of <u>levodopa</u> was unrelated to the time from levodopa initiation to motor complication onset. There no longer appears to be a rational basis for <u>delaying levodopa therapy in parkinsonian patients.</u> 2. Motor response complications reflect striatal system changes due to dopaminergic deafferentation and intermittent dopaminomimetic treatment and tend to normalize with the more physiologic stimulation afforded by continuous replacement strategies. 3. <u>Glutamatergic mechanisms affect extrapyramidal motor function:</u> In rats, D-2 mediated responses require concurrent NMDA receptor stimulation, while D-1 receptor-regulated pathways have varying degrees of sensitivity to NMDA receptor blockade; the subthalamic nucleus contributes primarily to the expression of D-2 mediated motor behaviors. In patients with Parkinson's disease, glutamatergic stimulation with milacemide transiently increased overall parkinsonian severity, especially rigidity. 4. No generalized defect in mitochondrial respiratory function could be documented in Parkinson's disease: oxygen consumption rates and respiratory chain enzyme activities in platelets and muscle mitochondria as well as blood lactate levels following glucose loading did not differ significantly between patients and controls. 5. Tourette syndrome patients have marked decreases in ventral brain areas, increases along the superior cortical convexities, and inverted relationships between these limbic and sensorimotor cortical regions appear on PET-fluorodeoxyglucose scans. 6. Physostigmine by continuous intravenous infusion at maximum tolerated levels inhibits CSF acetylcholinesterase by only 21% and produces little cognitive benefit. Amnesic doses of the anticholinergic, scopolamine, increase cortical function in normal elderly subjects in contrast to the decreases, especially parietotemporal, in PET scans of Alzheimer patients. Physostigmine, rather than normalizing, marginally diminishes cortical metabolism. 		

ANNUAL REPORT

October 1, 1991 through September 30, 1992

**Medical Neurology Branch
Clinical Neurosciences Program, DIR
National Institute of Neurological Disorders and Stroke**

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ANNUAL REPORT
October 1, 1991 through September 30, 1992

Medical Neurology Branch
Clinical Neurosciences Program, DIR
National Institute of Neurological Disorders and Stroke

Mark Hallett, M.D., Chief

The Medical Neurology Branch (MNB) differs from other branches in the Clinical Neurosciences Program (CNP). It has been the administrative organization for those sections not large enough or independent enough to be branches themselves. Components have come and gone frequently, and consequently, there never has been a single major scientific direction of the Branch.

Recently, concordant with the advice of the Board of Scientific Counselors, the Neuroimaging Section has become the Neuroimaging Branch, and the Clinical Epilepsy Section and the Neuronal Excitability Section have separated to become the Epilepsy Research Branch. The Clinical Neuropsychology Section was dissolved. Currently, the Medical Neurology Branch is composed of three relatively independent sections: the Cognitive Neuroscience Section (CNS), Jordan Grafman, Ph.D., Chief; the Neuromuscular Diseases Section (NDS), Marinos Dalakas, M.D., Chief; and the Human Motor Control Section (HMCS), Mark Hallett, M.D., Chief. This will be the constitution of the Branch for the immediate future.

While the Sections are independent, there are some areas of mutual interest. NDS and CNS have interests in AIDS and have been investigating some of the same patients. CNS has been collaborating in some of the studies of the HMCS relating to the role of the cerebellum in motor and cognitive function. All three sections are developing interests in the clinical problem of fatigue.

HUMAN MOTOR CONTROL SECTION

The Human Motor Control Section (HMCS) studies processes of control of normal human movement, deranged voluntary movement and involuntary movements. Patients studied include those with Parkinson's disease (PD), cerebellar deficits, dystonia, myoclonus, tremor, spinal cord injury and stroke. The work of the Section can be divided into the following subdivisions:

I. Voluntary movement physiology

A. Limb movement.

1. Multijoint coordination: This is a major area of research focused on the principles of movement involving more than one joint. Our hypothesis is that patients with cerebellar deficits will show abnormalities beyond what can be demonstrated with simple movements at single joints. We have already shown that there are deficits in performance that might be explained by a difficulty in timing of motor behavior or in producing the correct force. We are following up this problem by studying the perception of movement in patients with cerebellar deficits and the recruitment of motor units at the onset of a ballistic movement.

2. Motor skill learning: We have been trying to separate different processes that make up motor learning and are looking, particularly, to see if patients with cerebellar deficits might be deficient. In the last year, we have shown some problem with procedural learning in these patients.

3. Basic mechanisms and reflexes: Previously, we have done considerable work on spinal reflexes and found abnormalities in patients with movement disorders. This work continues, but we have added studies of muscle spindles. Using the technique of microneurography, we plan to investigate muscle spindle activity in patients with dystonia, PD, and cerebellar disorders.

B. Stance and gait

These important movements are studied as additional examples of multijoint movement. We have made some progress in looking at the movements that occur during quiet standing in aging and in patients with PD. We have begun to investigate locomotion in patients with cerebellar disorders.

II. Voluntary movement anatomy

A. Techniques.

We have focused a major effort in this area in order to be able to identify areas of the brain active with voluntary movement. The plan is to map the results of each modality onto the magnetic resonance imaging (MRI) scan. Magnetic stimulation can map the primary motor cortex. Positron emission tomography (PET) can map all areas of brain that are active. Movement-related cortical potentials (MRCPs) can give precise timing information as to when these areas are active. The electrophysiologic data is mapped into the MRI by obtaining a 3-D digitization of the head. We are just beginning to obtain successful results..

1. Transcranial magnetic stimulation (TMS): This is the most active area of research in HMCS. We have been able to map motor representations in the cortex -- excitatory, inhibitory, contralateral and ipsilateral. We have shown that the excitatory and inhibitory areas differ. Additionally, the sites that provoke movement differ from those that can block the perception of somatosensory stimuli. We are now developing methodology for rapid rate magnetic stimulation (rTMS). We have begun to understand the complex of excitatory and inhibitory effects produced by multiple stimuli and are developing safety standards for use of the rTMS device.

2. PET: Technical advances in PET have allowed us to superimpose PET on the MRI and to do statistical mapping using the Hammersmith programs. We are beginning to map brain regions active in different movements. We have made progress in the topography of movement in different parts of the brain.

3. Movement-related cortical potentials (MRCPs): With attention paid to the potentials around the time of movement onset, we have made a new comprehensive proposal as to the sources of these potentials and are trying to relate these to the known anatomy. An important step has been developing a model of MRCPs with dipoles that can explain the EEG potentials. We have done this on the basis of biological knowledge and have been successfully comparing the locus of these dipoles to areas seen to be activated with PET studies.

B. Physiologic problems

1. Plasticity: Our group has made the first clear observations showing plasticity of the motor cortex in humans. We have studied amputation, spinal cord injury, hemispherectomy, anesthesia of a limb, and the reading finger of Braille readers. We have found that some plastic changes can occur within minutes. Additionally, we have localized some of the change to intracortical connections. We plan to study stroke.

2. Excitatory and inhibitory effects: We have mapped inhibitory effects of TMS as well as excitatory effects. Our observations have given the first clear demonstration of active ipsilateral pathways in normal humans. We have found that TMS can speed up reaction time as well as prolong it, both in normal subjects and those with Parkinson's disease. This might even have therapeutic utility. TMS can influence the voluntary choice of which movement to make and can be used to analyse other aspects of motor physiology. We have also developed a new method for analysis of central fatigue.

III. Studies of involuntary movement

This aspect of our work is becoming less emphasized. Our studies have focused on the pathophysiology of these disorders.

A. Myoclonus

We have confirmed a recent observation that some patients with tremor have a myoclonic disorder, and we have studied this physiology. We have also characterized twitching movement of the face in patients with olivopontocerebellar atrophy and found it to be a facial action myoclonus.

B. Tremor

We have conducted studies confirming and further characterizing the central generators of essential tremor and parkinsonian tremor. In a PET study of essential tremor, we have shown that the cerebellum and its connections appear to play a significant role.

C. Palatal tremor (palatal myoclonus)

Using clinical, MRI and physiologic studies, we have shown that this disorder should be divided into two distinct disorders, called essential palatal tremor and symptomatic palatal tremor.

IV. Therapeutic studies.

These studies are kept as a side interest. They are valuable to attract patients to participate in our physiologic work. We also like to be able to treat our patients to the extent that this is possible.

A. Botulinum toxin for focal dystonias.

This was a very active area, but is now being deemphasized. Our group was the first to show the utility of botulinum toxin for focal hand dystonias, and we are making continuing observations about the long term utility. Together with Dr. Ludlow's group in NIDCD (which used to be a part of the HMCS), we have been the first to show the utility of botulinum toxin type F for patients who have developed antibodies to type A.

B. Therapeutic trials for cerebellar ataxia.

We have tried to find some medication that will benefit ataxia for the large number of patients with cerebellar deficits that we see. Our trial using 5-hydroxytryptophan (HTP) was aborted because of a probable case of eosinophilia-myalgia-syndrome. In its place, we are initiating a trial of busparone, a selective 5-HT_{1A} agonist.

COGNITIVE NEUROSCIENCE SECTION

The primary objectives of the Cognitive Neuroscience Section are to identify and model the components of information processing, the cognitive computations that underlie each component, and the categories and architecture of knowledge representation systems. Furthermore, we make an effort to map knowledge networks and cognitive processes onto human brain physiology, structures, and systems.

Investigators in the Cognitive Neuroscience Section are currently studying a wide range of cognitive processes including planning and reasoning in patients with prefrontal lobe dysfunction; amnesia, memory, and knowledge representation; number processing and calculation; object recognition and naming; the relationship of emotion and mood state to cognitive processing, and visual perception. Although many of our studies utilize young and old normal subjects, the majority of our studies are conducted with central nervous system (CNS) impaired adult patients. CNS impaired patients are useful to study because their cognitive deficit pattern often implies dissociations between types of information processing components, cognitive computational properties, or knowledge domains. Thus, not only can such patients teach us about the structure of cognition on the basis of their performance dissociations (and associations, too), but the nature and direction of the dissociations may lead to inferences regarding the contribution of regional neural networks and neurochemical systems to cognitive processing. We utilize both single-case within-subject and traditional group-comparison designs in our research.

The methodologic approach to studying the components of information processing has been successfully applied to several lines of research in the Cognitive Neuroscience Section. For example, the study of memory in multiple sclerosis patients has narrowed an aspect of their information processing deficit to two components: the articulatory rehearsal loop component of working memory which temporarily stores and refreshes phonologic information when it cannot be processed on-line, and a post-rehearsal retrieval pathway. Dr. Ray Johnson, Jr., Head of the Cognitive Neurophysiology Unit, using event-related potential methodology, has demonstrated that working memory rehearsal loops can be reflected in event-related slow waves and that these rehearsal loops, while equally sensitive to information load, have different time courses and distinct topographic distributions. Additional work in his Unit by Dr. Marten Scheffers has demonstrated that patients with chronic fatigue syndrome demonstrate normal sensitivity and accuracy on highly demanding visual search and attention tasks. They did, however, have slowed reaction times. This work has narrowed the search for the locus of their reported central fatigue to performance execution processes. Other work by Dr. Ray Johnson, Jr. has focused on using event-related potentials to disambiguate the memory and information processing deficits described in various cortical and subcortical dementias. Other work in the Section has utilized an error-analysis approach to the performance of CNS impaired patients in order to better characterize the computational processes that underlie cognition. For example, Dr.

Paolo Nichelli is studying time perception and estimation of time duration in patients and normal controls. Subjects have to estimate elapsed time on psychophysical judgment tasks. His results, based on error-analyses, indicate that there are multiple "clocks" underlying time estimation including clocks related to temporal order, temporal binding, short-term (<30 seconds) and long-term time duration estimation. Dr. Nichelli is also preparing studies to componentially analyze planning performance in chess. Dr. Ildebrando Appollonio is comparing the performance of patients with Parkinson's disease (PD) and cerebellar atrophy on various memory and cognitive tasks. His analysis of their error performance is helping narrow down the reason for patient failures in different aspects of memory performance. In particular, his analyses focus on the role of intentional and goal-related behaviors in impaired encoding and retrieval of information in PD and cerebellar atrophy. Another ongoing analysis of PD patients focuses on their visual processing. Dr. Mark Beeman has conducted a set of lexical processing studies designed to examine the role of the right hemisphere in word processing. On the basis of an error analysis, Dr. Beeman has hypothesized that the right hemisphere plays an important role in the connotative analysis of words. Finally, Drs. Grafman, Nichelli, Beeman and Appollonio are using priming methodology in tasks requiring word and object recognition in order to map out the architecture of various domains of knowledge representation.

As a result of these and other studies in progress, we have been able to tentatively assign cognitive components and knowledge representation systems to brain locations. For example, structural analysis of visual stimuli takes place in the posterior cortex in regions distinct from where meaningful analysis of stimuli takes place. In addition, the more complex the nature of stimulus representation (e.g., schemas), the more anterior in the brain is its representation. Representational systems are organized both serially and in parallel, can be activated in parallel, are partially information redundant, and while informationally hierarchical, can be activated selectively via attentional mechanisms. Basal ganglia structures appear to aid in the execution of representations (e.g., via motor procedures or cognitive planning). The involvement of many different neural structures and neurotransmitter systems in cognition are currently being studied. While we are concerned with specifying the cognitive components of specific neural systems and structures, we also expect in the next few years to describe more general principles of cognitive processing and representational knowledge and to map these broad principles to brain chemo- and neuroanatomy. As alluded to above, specific projects carried out in the Cognitive Neuroscience Section have supported the conceptual distinction between cognitive components, computational properties, and knowledge domains that are at the heart of the neuropsychologic models developed or tested by Cognitive Neuroscience Section members.

NEUROMUSCULAR DISEASES SECTION

The Neuromuscular Diseases Section conducts clinical studies and laboratory investigations to determine etiology (infection/immunity and/or genetics) of patients with neuromuscular disorders and explore new therapies. Current studies include: (1) motor neuron diseases syndromes such as amyotrophic lateral sclerosis (ALS) and post-polio syndrome; (2) demyelinating polyneuropathies; (3) inflammatory myopathies; (4) neuromuscular diseases associated with HIV infection; (5) experimental models of retrovirus-induced polymyositis; (6) mechanisms of muscle regeneration; (7) studies involving the infection of muscle or Schwann cells in tissue culture with viruses, especially HIV and enteroviruses; (8) study of fatigue and

metabolic myopathies with ^{31}P magnetic resonance spectroscopy; and (9) clinical experimental therapeutic studies in patients with post-polio syndrome, polymyositis, demyelinating polyneuropathies, and HIV-related neurologic diseases.

We have found that patients with the post-polio syndrome have an increase association with HLA DQ17 haplotype suggesting genetic susceptibility to the polio infection. Phenotypically abnormal subpopulations of lymphocytes also have been noted in some of these patients. These, along with the presence of inflammation in the muscle biopsy and spinal cord and oligoclonal IgG bands in the cerebrospinal fluid (CSF), prompted a double-blind placebo control study using prednisone. The results of this study are now being analyzed.

We have defined the spectrum of neuromuscular diseases associated with HIV infection. Study of these patients' muscle biopsies using immunocytochemistry, in situ hybridization, polymerase chain reaction (PCR) and tissue culture, showed viral antigens only in occasional endomysial lymphoid cells but not within the muscle fibers themselves. Studies of human myotubes in culture showed that retroviruses or retrovirus-infected macrophages cannot infect or replicate within the muscle myotubes. On the basis of these studies and the characterization of endomysial lymphocyte subsets, we have concluded that HIV-polymyositis is not due to direct infection of the muscle by the virus but rather due to a T-cell mediated and MHC-1-restricted cytotoxic process, triggered by the virus. These findings, which were identical to those seen in HIV-negative polymyositis, were also noted in polymyositis associated with HTLV-I infection.

We have searched for enteroviruses, retroviruses, coxsackie virus, mumps and adenoviruses in the muscles of patients with inflammatory myopathies using PCR and specific primers. We failed to detect viral RNA in any of the muscle specimens.

Human myotubes express the poliovirus receptor and are susceptible to direct infection with the virus. By PCR and electron microscopy, the virus was found within the myotubes. By contrast, human myotubes that lack the CD4 receptor, were resistant to infection with HIV, HTLV-I or with macrophages infected with these viruses.

We have found that the drug AZT currently used in the treatment of AIDS, can cause a destructive mitochondrial myopathy. The AZT-induced abnormalities in the muscle mitochondria have been demonstrated by light microscopy, electron microscopy, immunocytochemistry using antibodies to double-stranded DNA, and with molecular studies that showed 80% depletion of the muscle mitochondrial DNA. AZT in tissue culture of human muscle was also toxic to mitochondria and, when injected into rats, caused morphologic and functional changes in the mitochondria of both skeletal and cardiac muscles.

The IgM paraprotein associated with demyelinating polyneuropathy was found to be an antibody against peripheral nerve glycolipids and sulfatides. The glycolipid GD_{1b} was found to immunoreact with the serum IgM and may serve as antigen in these neuropathies.

The cellular events of muscle fiber regeneration were examined using monoclonal antibodies that recognize satellite muscle cells. We found that neural cell adhesion molecules (N-CAM) and Leu-19, a marker for NK cells, are common antigens shared by regenerating muscle fibers, satellite cells and lymphoid tissue. The usefulness of

these markers in studying the mechanism of muscle regeneration in various neuromuscular disorders is being studied.

The poliovirus receptor was immunolocalized at the endplate and in some degenerating muscle fibers. The role of the muscle as a potential route of entry of the poliovirus into the CNS via the endplate and through retrograde transport is now being examined.

We have explored a series of new therapies in patients with the post-polio syndrome, demyelinating polyneuropathies and inflammatory myopathies refractory to available immunotherapies. Controlled, randomized studies are conducted with combination chemotherapy and with high-dose intravenous immunoglobulin (IVIg). Preliminary findings indicate that IVIg is effective in treating patients with dermatomyositis and paraproteinemic polyneuropathies. A double-blind placebo-control trial using prednisone to treat post-polio syndrome is near completion. A study with IVIg in patients with ALS is also near completion. A pilot trial using continuous intrathecal AZT infusion for patients with AIDS-dementia complex is now ready to begin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02667-08 MNB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Analysis of Involuntary Movements

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Mark Hallett, M.D.	Clinical Director	OCD	DIR	NINDS
	Chief	HMCS	MNB	DIR NINDS
Others: Camilo Toro, M.D.	Visiting Associate	HMCS	MNB	DIR NINDS
Joseph Matsumoto, M.D.	Special Volunteer	HMCS	MNB	DIR NINDS
Gunther Deuschl, M.D.	Special Volunteer	HMCS	MNB	DIR NINDS
Barbara Karp, M.D.	Chief, Consultation Service	OCD	DIR	NINDS
Josep Valls-Sole, M.D., Ph.D.	Visiting Associate	HMCS	MNB	DIR NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Human Motor Control Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	3.0	PROFESSIONAL:	2.5	OTHER:	0.5
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CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Involuntary movements have often been difficult to classify clinically. Clinical and physiologic analysis of a continuing series of patients has led to new classifications and pathophysiologic insights.

Patients with myoclonus have been studied to seek further understanding of this confusing involuntary movement. Extensive studies have classified most cases seen within previously identified categories. We have studied and characterized physiologically three patients with tremor that have had cortical myoclonus and fit the disorder of cortical tremor.

Physiologic studies of essential tremor and parkinsonian tremor using transcranial magnetic stimulation have shown that both tremors can be reset by direct brain stimulation. This gives further information about the nature of the central generators of these tremors.

Extensive clinical and physiologic studies have been completed in patients with palatal tremor (myoclonus). We have further data confirming the division of these patients into two groups: idiopathic and secondary.

A study of movement-related cortical potentials in patients with dystonia (hand cramps) have revealed an abnormality of cortical activation.

A physiologic investigation of facial twitching seen in patients with olivopontocerebellar atrophy has revealed that this disorder is a form of myoclonus. The disorder probably results from brain stem degeneration.

Positron emission tomography (PET) studies of essential tremor using region cerebral blood flow have confirmed our earlier observations using glucose metabolism and implicate the cerebellum and its pathways in the genesis of this disorder.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02669-08 MNB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Physiological Analysis of Voluntary Movement		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.: Mark Hallett, M.D.	Clinical Director	OCD DIR NINDS
	Chief	HMCS MNB DIR NINDS
Others: Camilo Toro, M.D.	Visiting Associate	HMCS MNB DIR NINDS
Thomas Zeffiro, M.D., Ph.D.	Sr. Staff Fellow	HMCS MNB DIR NINDS
Steve Grill, M.D.	Clinical Associate	HMCS MNB DIR NINDS
Jau-Shin Lou, M.D.	Clinical Associate	HMCS MNB DIR NINDS
Maria Lebiedowska, M.D., Ph.D.	Special Volunteer	HMCS MNB DIR NINDS
COOPERATING UNITS (if any) Department of Rehabilitation Medicine, Clinical Center Department of Nuclear Medicine, Clinical Center		
LAB/BRANCH Medical Neurology Branch, CNP, DIR		
SECTION Human Motor Control Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 6.5	PROFESSIONAL: 5.0	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Studies of <u>voluntary movement</u> focused on the role of the <u>cerebellum</u> . One issue was the contribution of the cerebellum to <u>coordination</u> . The results seem to indicate that the cerebellum is critical for the coordination of multijoint movement. One role of the cerebellum appears to be in the control of <u>force</u> . A second issue is the role of the cerebellum in <u>motor learning</u> . In tasks of <u>motor learning</u> , it has been demonstrated that patients with cerebellar disturbances have difficulty with procedural visuomotor learning. Patients with <u>Parkinson's disease (PD)</u> had normal procedural learning. A third issue is the role of the cerebellum in the sense of movement. Results so far show a deficit in appreciating the size of movements in patients with cerebellar deficits. Using O-15 labelled water as a marker for cerebral blood flow in <u>positron emission tomography (PET) studies</u> , we have been working on methods for improved anatomic correlation of regions of metabolic change by superimposing the PET image onto an MRI image. Regions of the brain activated with voluntary movement and vibration of the hand have been identified and quantified. Special attention is being paid to the topography of activation in different parts of the brain including the cerebellum. A special study was devoted to the role of the cerebellum in learning of a sequence of finger movements. Studies of <u>movement-related cortical potentials</u> have focused on identifying <u>dipoles</u> for the generation of the different components and the development of techniques for measuring event-related desynchronization of the EEG. The dipoles have been compared with areas of activation with PET and an excellent correlation has been found. Studies have been completed in patients with <u>dystonia</u> that show reduction in amplitude of some components. Studies in the Biomechanics Laboratory of the Department of Rehabilitation Medicine have focused on the control of <u>balance and gait</u> . A study is in progress of gait in patients with cerebellar disorders. Other studies are being done of balance in patients with cerebellar deficits. New studies have been initiated in recording <u>muscle spindle</u> activity during voluntary movement.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02711-07 MNB
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PERIOD COVERED October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Utility and Physiology of Botulinum Toxin for Involuntary Movement Disorders
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PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)					
P.I.:	Mark Hallett, M.D.	Clinical Director	OCD	DIR	NINDS
		Chief	HMCS	MNB	DIR NINDS
Others:	Barbara I. Karp, M.D.	Chief, Consultation Service	OCD	DIR	NINDS
	Leo Cohen, M.D.	Visiting Scientist	HMCS	MNB	DIR NINDS
	Stephen Grill, M.D., Ph.D.	Clinical Associate	HMCS	MNB	DIR NINDS
	Jau-Shin Lou, M.D., Ph.D.	Clinical Associate	HMCS	MNB	DIR NINDS

COOPERATING UNITS (if any) Speech Pathology Unit, NIDCD

LAB/BRANCH Medical Neurology Branch, CNP, DIR

SECTION Human Motor Control Section

INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: .5	PROFESSIONAL: 0.3	OTHER: 0.2
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CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have been studying the efficacy of local injections of <u>botulinum toxin</u> for the treatment of different types of <u>focal dystonias</u> . Botulinum toxin injected in small doses directly into muscle, binds to the <u>neuromuscular junction</u> and inactivates it for approximately three months. We have also been using botulinum toxin to study the physiology of focal dystonias. Studies of the utility of botulinum toxin are being carried out in <u>writer's cramp</u> (and its variants such as pianist's cramp) in open-label and double-blind trials. <u>Treatment</u> appears effective at least in the short-term. Longer follow-up on our patients is now being analyzed. In our subgroup of patients with musician's cramp, most of the patients eventually decide the treatment is insufficient for their needs. We are conducting a phase I trial of botulinum toxin type F to see if this will benefit patients who have developed antibodies to type A. In the first 16 patients, it appears to have similar efficacy and side effects to type A.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02712-07 MNB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Noninvasive Stimulation of Human Central Nervous System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	Mark Hallett, M.D.	Clinical Director Chief HMCS MNB DIR NINDS
Others:	Leo Cohen, M.D. A. Pascual-Leone, M.D., Ph.D. Joachim Brasil-Neto, M.D. Josep Valls-Sole, M.D., Ph.D. Eric Wassermann, M.D.	Visiting Scientist Clinical Associate Visiting Fellow Visiting Associate Clinical Associate HMCS MNB DIR NINDS HMCS MNB DIR NINDS HMCS MNB DIR NINDS OCD DIR NINDS
COOPERATING UNITS (if any) Speech and Voice Pathology Unit, NIDCD		
LAB/BRANCH Medical Neurology Branch, CNP, DIR		
SECTION Human Motor Control Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS	4.5	PROFESSIONAL: 4.0 OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Recently, techniques have become available for the <u>noninvasive stimulation</u> of the <u>human cortex</u> and deep proximal <u>peripheral nerves</u>. Stimulation can be with a high-voltage, extremely brief <u>electrical</u> pulse or with <u>magnetic stimulation</u>. One purpose is to use these methods for noninvasive localization of different parts of the human cortex including motor cortex, sensory cortex and language cortex. Another purpose is to study <u>cortical physiology</u> in different disease states.</p> <p>We have continued to make advances in understanding the technical aspects of magnetic stimulation, defining the optimal method to map different body part representations in the <u>motor cortex</u>. We have been able to compare the site of stimulation with the area of the brain activated in positron emission tomography (PET) scans by mapping the results of both studies onto the same magnetic resonance imaging scan; correlation is excellent. Extensive effort has been devoted to the study of the <u>inhibitory effects</u> of brain stimulation. We have shown, for example, that the ipsilateral silent period does not appear to be transmitted by the corpus callosum. We have also studied the response of the brain to pairs and trains of stimuli. The brain undergoes a complex response of excitation and inhibition. With long and strong trains, epileptic seizures can be precipitated, and we have developed safety guidelines to avoid this. We have studied <u>reaction time</u> to brain stimulation and found that magnetic stimulation can speed the response to any other stimulus. Magnetic stimulation can also speed the responses in patients with Parkinson's disease, and this effect may be useful therapeutically. Following-up our previous studies that showed <u>reorganization</u> of motor cortex pathways following anesthetic block of the forearm and hand, we have shown that this effect is probably mediated largely at the cortical level.</p> <p>In relation to sensory effects, we have demonstrated that the sites of stimulation that block somatosensory sensation differ from those responsible for motor activation. We have continued to study the <u>visual cortex</u> demonstrating that it was possible to produce brief visual images in <u>blind patients</u>. This technique may identify patients suitable for a <u>visual prosthesis</u> that uses direct brain stimulation.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02792-04 MNB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neuropsychological Investigations of Human Cognition and Mood State		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Jordan Grafman, Ph.D. Chief Others: Ray Johnson, Jr., Ph.D. Special Expert Paolo Nichelli, M.D. Visiting Scientist Mark Beeman, Ph.D. IRTA Fellow Ildebrando Appollonio, M.D. Special Volunteer Mark Hallett, M.D. Chief	CNS MNB NINDS CNS MNB NINDS CNS MNB NINDS CNS MNB NINDS CNS MNB NINDS MNB NINDS	
COOPERATING UNITS (if any)		
LAB/BRANCH Medical Neurology Branch, CNP, DIR		
SECTION Cognitive Neuroscience Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Current studies in the Cognitive Neuroscience Section focus on <u>amnesia</u> , <u>thinking</u> , <u>neurolinguistics</u> , <u>event-related evoked potentials</u> , <u>social cognition</u> , and <u>visual processing</u> . Both single-case and group design studies are used. Normal controls, inpatients and outpatients are evaluated. <u>Memory</u> is studied in experiments focusing on implicit and explicit retrieval, priming, autobiographical recall, discourse processing, naming and word retrieval, and categorization tasks. <u>Reasoning</u> and <u>problem-solving</u> are studied in experiments focusing on planning, syllogisms, analogical thinking, and schema organization. <u>Dyslexia</u> , <u>dysgraphia</u> , and <u>dysnomia</u> , are studied in experiments focusing on single word reading and writing, lexical decision, associative and semantic priming, and similar tasks. <u>Event-related evoked potentials</u> are measured for latency, amplitude, and distribution and used as a physiologic index of information-processing stages, working memory, visual attention, and automatic processing. <u>Emotions</u> , <u>impression</u> and <u>preference formation</u> , and social judgment are studied in experiments focusing on judgment of interpersonal behavior, word association, and mood state. Finally, <u>visual information processing</u> is studied, beginning with experiments examining spatial frequency contrast-sensitivity, object recognition, and visual categorization. Although developing theoretically valid and testable models of cognitive processing is the primary aim of the Section, there is also a strong effort to relate the profile of cognitive deficits in patients to lesion location in order to topographically map the components of cognitive processing to brain regions and systems. <u>Pharmacologic challenge</u> and infusion studies are planned to evaluate the dissociability of hypothesized components of memory processing. <u>MRI functional stimulation</u> and <u>PET scan studies</u> are planned to examine whether plans are processed in a unique brain location.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02793-04 MNB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cognitive Neuroscience

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jordan Grafman, Ph.D.	Chief	CNS	MNB	NINDS
Others:	Ray Johnson, Jr., Ph.D.	Special Expert	CNS	MNB	NINDS
	Mark Beeman, Ph.D.	IRTA	CNS	MNB	NINDS
	A. Salazar, M.D.	Department of Neurology, Walter Reed Army Med. Ctr.			
	S. Rao, Ph.D.	Dept. of Neurology, Medical College of Wisconsin			
	F. Boller, Ph.D.	INSERM U. 324 Centre Paul Broca, Paris, France			
	*				

COOPERATING UNITS (if any)

Walter Reed Army Medical Center, Wash, DC; National Naval Medical Center, Bethesda, MD; Centre Paul Broca, Paris, France; Hopital Salpetriere, Paris, France; Hospital Clinicas, Montevideo, Uruguay; **

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Cognitive Neuroscience Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.4

PROFESSIONAL:

0.2

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Memory and cognition are studied in experiments focusing on representational knowledge, working memory, priming, procedural learning, number processing and calculation, autobiographical memory, visual attention, naming, and categorization. Normal subjects and patients with progressive dementia, focal lesions, and psychiatric disorders are studied. New studies focusing on the composition of mental structures in the frontal lobes have just begun.

*Continued:

Y. Agid, M.D.	INSERM U. 289 Hopital Salpetriere, Paris, France
A. Sirigu, Ph. D.	INSERM U. 289 Hopital Salpetriere, Paris, France
B. Dubois, M.D.	INSERM U. 289 Hopital Salpetriere, Paris, France
J. Hallenbeck, M.D.	Chief, Stroke Branch, NINDS
E. Zaidel, Ph.D.	Dept. of Psychology, UCLA, Los Angeles, CA
C. Junque, Ph.D.	Dept. of Neurology, Hosp. St. Pau, Barcelona, Spain
J. Hendler, Ph.D.	Dept. of Computer Science, University of Maryland
K. Holyoak, Ph.D.	Dept. of Psychology, UCLA, Los Angeles, CA

** Medical College of Wisconsin, Milwaukee, Wisconsin; National Institute of Mental Health, NIH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 N5 02794-04 MNB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Event-Related Potential Studies of Normal and Abnormal Cognitive Processing		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Ray Johnson, Jr., Ph.D.	Special Expert CNS MNB DIR NINDS
Others:	Marten Scheffers, Ph.D.	Visiting Fellow CNS MNB DIR NINDS
	Jordan Grafman, Ph.D.	Chief CNS MNB DIR NINDS
	Daniel Ruchkin, Ph.D.	Elec. Engineer U. of Maryland School of Medicine
	Wolfgang Miltner, Ph.D.	Psychologist U. of Tuebingen, Germany
COOPERATING UNITS (if any) University of Maryland School of Medicine, University of Tuebingen, Germany		
LAB/BRANCH Medical Neurology Branch, CNP, DIR		
SECTION Cognitive Neuroscience Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS:	2.0	PROFESSIONAL: 2.0 OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Event-related brain potentials</u> (ERP) were used to study <u>cognitive processes</u> such as short- and long-term memory, spatial attention and visual search, mental rotation, mental arithmetic, and language comprehension. ERP studies of normal subjects were intended to reveal the brain mechanisms underlying cognition. Studies of patients with <u>neurologic disorders</u> were intended to allow us to characterize better patients' information processing deficits while providing information on the physiologic mechanisms underlying these cognitive processes. Data collection was completed in ERP studies of dementia (<u>Alzheimer's and Parkinson's diseases</u>, <u>HIV disease</u>, and <u>progressive supranuclear palsy (PSP)</u>). The results from the HIV and PSP studies indicate that, in the earliest stages of subcortical disease, processing at the cortical level is more affected than processing at the subcortical level, (i.e., resembling a cortical dementia). This pattern reverses as the subcortical disease progresses. A follow-up study on the modality specificity of deficits in PSP patients has been completed. Studies of the mechanisms underlying and affecting <u>attentional processes</u> continue and data analysis is complete in two studies, one on how normal controls visually search a spatial array for items previously stored in short-term memory, and one on the effects of <u>fatigue</u> on attention in patients with <u>chronic fatigue syndrome (CFS)</u> and normal controls. Studies with Dr. Daniel Ruchkin have demonstrated that memory rehearsal processes are marked by large negative slow waves and that different brain areas are invoked to perform verbal and spatial short-term memory rehearsal processes. Additional studies on the nature of short- and long-term memory deficits in <u>amnesic</u> patients and <u>multiple sclerosis</u> patients have been completed and data analysis has begun. Data analysis continues on studies of <u>temporal lobectomy</u> patients, <u>Turner's</u> patients, and the maturation of cognitive processes. Studies with Dr. Wolfgang Miltner have been aimed at providing additional data on the neural generator mechanisms underlying cognitive processes. Patient and control data have been used to validate the predictions of Johnson's model of the variables controlling P300 amplitude. These data revealed that, contrary to the widely accepted notion, the P300 is a component whose amplitude represents the simultaneous utilization of a number of cognitive processing "modules." During recognition memory in controls and patients, these modules appear to indicate the presence and functioning of different memory systems. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02038-20 MNB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Combined Clinical, Viral and Immunological Studies of Neuromuscular Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: M.C. Dalakas, M.D., Chief, NDS, MNB, DIR, NINDS Other: <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> I. Illa, M.D., Neurologist, NDS, MNB, DIR, NINDS R. Quarles, Ph.D., Biochemist, DMN, DIR, NINDS M. Monzon, Ph.D., Special Expert, NDS, MNB, DIR, NINDS D. Stein, M.D., Neurologist, NDS, MNB, DIR, NINDS B. Sonies, Ph.D., Speech Pathologist, CC, DIR, NINDS M. Ropka, M.D., Director, IP, NR </div> <div style="width: 45%;"> M. Agboatwalla, M.D., Child Specialist, Karachi, Pakistan A. McLaughlin, Ph.D., DRRP, OD </div> </div>		
COOPERATING UNITS (if any)		
LAB/BRANCH Medical Neurology Branch, CNP, DIR		
SECTION Neuromuscular Diseases		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Clinical</u> and <u>laboratory</u> studies are conducted to determine etiology (<u>infection</u>, <u>immunity</u> and/or <u>genetics</u>) of chronic diseases of the neuromuscular system and design effective therapies. Current studies involve patients with <u>polymyositis/dermatomyositis (PM/DM)</u>, <u>post-polio syndrome</u>, <u>amyotrophic lateral sclerosis (ALS)</u>, <u>demyelinating polyneuropathies</u>, <u>neuromuscular diseases</u> associated with HIV infection, and <u>hypokalemic periodic paralysis</u>. </p> <p> The pathogenesis of post-polio syndrome is explored with a series of electrophysiologic, virologic, immunologic and histologic studies. The findings are compared with those seen in patients with <u>acute paralytic poliomyelitis</u> and other <u>motor neuron diseases</u>. Persistent or mutant poliovirus is sought in these patients' tissues using tissue cultures, polymerase chain reaction (PCR), and <u>in situ</u> hybridization. Because abnormal immunoregulation was found in some patients, a double-blind placebo-controlled trial using prednisone is conducted. The mechanism of <u>post-polio fatigue</u>, a common and disabling symptom in many patients, is under study. The spectrum of <u>neuromuscular disorders</u> associated with <u>HIV</u> infection has been studied and the role of the virus as the cause of <u>neuropathy</u> or <u>myopathy</u> is investigated with a variety of immunocytochemical studies, <u>in situ</u> hybridization and PCR. The antiretroviral drug AZT was found to cause a specific myopathy with abnormal mitochondria on the basis of various morphologic, molecular, biochemical and immunocytochemical studies. A longitudinal study of HIV-positive patients that develop myopathic symptoms while on AZT is conducted with serial muscle biopsies to assess factors associated with the development of myopathy and design effective therapies. The metabolic basis of fatigue is studied in patients with <u>mitochondrial myopathies</u>, post-polio syndrome, <u>chronic fatigue syndrome</u> and HIV- or AZT-associated myopathies using exercise magnetic resonance spectroscopy. </p> <p> Randomized-controlled clinical trials are conducted with high-dose intravenous immunoglobulin (IVIg) in patients with <u>PM/DM</u>, <u>chronic inflammatory</u> and <u>paraproteinemic demyelinating polyneuropathies</u> and <u>ALS</u>. A controlled study using <u>dichlorophenamide</u>, a carbonic anhydrase inhibitor, is ready to begin in patients with hypokalemic periodic paralysis. </p>		
15-MNB/DIR		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02531-11 MNB			
PERIOD COVERED October 1, 1990 through September 30, 1991					
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies in Neuromuscular and CNS Diseases and Their Experimental Models					
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> PI: M.C. Dalakas, M.D. OTHERS: M. Monzon, Ph.D. L. Lamperth, M.D. I. Illa, M.D., Ph.D. R. Quarles, Ph.D. A.A. Ilyas, M.D. </td> <td style="width: 33%; vertical-align: top;"> Chief, NDS Special Expert, NDS Visiting Associate, NDS Visiting Associate, NDS Biochemist Biochemist </td> <td style="width: 33%; vertical-align: top;"> MNB, DIR, NINDS MNB, DIR, NINDS MNB, DIR, NINDS MNB, DIR, NINDS DMN, DIR, NINDS N.J. Medical School </td> </tr> </table>			PI: M.C. Dalakas, M.D. OTHERS: M. Monzon, Ph.D. L. Lamperth, M.D. I. Illa, M.D., Ph.D. R. Quarles, Ph.D. A.A. Ilyas, M.D.	Chief, NDS Special Expert, NDS Visiting Associate, NDS Visiting Associate, NDS Biochemist Biochemist	MNB, DIR, NINDS MNB, DIR, NINDS MNB, DIR, NINDS MNB, DIR, NINDS DMN, DIR, NINDS N.J. Medical School
PI: M.C. Dalakas, M.D. OTHERS: M. Monzon, Ph.D. L. Lamperth, M.D. I. Illa, M.D., Ph.D. R. Quarles, Ph.D. A.A. Ilyas, M.D.	Chief, NDS Special Expert, NDS Visiting Associate, NDS Visiting Associate, NDS Biochemist Biochemist	MNB, DIR, NINDS MNB, DIR, NINDS MNB, DIR, NINDS MNB, DIR, NINDS DMN, DIR, NINDS N.J. Medical School			
COOPERATING UNITS (if any)					
LAB/BRANCH Medical Neurology Branch, CNP, DIR					
SECTION Neuromuscular Diseases					
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892					
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5			
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </td> <td style="width: 33%; vertical-align: top;"> <input checked="" type="checkbox"/> (b) Human tissues </td> <td style="width: 33%; vertical-align: top;"> <input type="checkbox"/> (c) Neither </td> </tr> </table>			<input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The Section runs the Laboratory of <u>Muscle Enzyme Histochemistry</u> that processes up to 300 muscle and nerve biopsies per year for diagnostic studies. Examined muscle specimens are from patients with neuromuscular manifestations related to systemic <u>autoimmune</u>, <u>viral</u>, <u>metabolic</u>, <u>endocrine</u> or <u>infectious diseases</u>, and from patients with primary neuromuscular disorders, such as <u>polymyositis</u>, <u>dermatomyositis</u>, <u>neurogenic muscular atrophies</u>, <u>muscular dystrophies</u>, <u>post-polio syndrome</u>, <u>polyneuropathies</u>, <u>mitochondrial encephalomyopathies</u> and <u>biochemical</u> or <u>genetic</u> muscle diseases. The laboratory is also involved in the following immunologic, biochemical and virologic studies that examine the susceptibility of the muscle and nerve to immune or viral mediated injuries: (a) Study of the regeneration of human muscle in health and disease, and monitor the maturation of <u>satellite cells</u> by examining the expression of neural cell adhesion molecules and laminins; (b) study the susceptibility of muscle and nerve to <u>infection with retroviruses</u> and the ability of HIV or HIV-infected lymphoid cells to infect human myotubes in culture and induce expression of MHC-antigens; (c) study the expression of the <u>poliovirus receptor</u> in human muscle and the ability of the <u>poliovirus</u> to infect and replicate in human myotubes; (d) study the <u>toxicity of AZT to muscle mitochondria</u> by applying various concentrations of AZT to human muscle in culture; and (e) use of <u>animal models</u> to study the pathogenesis of: (i) <u>retrovirus-induced inflammatory myopathy</u> by examining muscles from monkeys infected with <u>simian immunodeficiency virus</u>; (ii) AZT-induced mitochondrial myopathy by studying structural, metabolic and functional changes in the muscle, heart, and brain <u>mitochondria</u> in rats injected with AZT; and (iii) <u>autoimmune demyelinating polyneuropathies</u> by searching for myelinotoxic or axonotoxic autoantibodies in sciatic nerves of rats after intraneural injection of the patient's serum. </p>					

ANNUAL REPORT
October 1, 1991 through September 30, 1992

STROKE BRANCH
Clinical Neurosciences Program, DIR
National Institute of Neurological Disorders and Stroke

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ANNUAL REPORT
October 1, 1991 through September 30, 1992
Stroke Branch
Clinical Neurosciences Program, DIR
John M. Hallenbeck, M.D., Chief

The Stroke Branch is composed of three sections, Clinical Investigation, Cerebrovascular Pathophysiology and Neurocytobiology. The research in these sections is integrated and directed toward the goal of developing measures to reduce stroke risk and to minimize tissue damage and optimize functional recovery in the event a stroke occurs.

Both clinical and basic stroke research are carried out in the Clinical Investigation Section. Dr. Tom DeGraba and a patient coordinator, Paula Oberlander, were hired during the summer of 1992, and they are initiating clinical research programs at the National Naval Medical Center under the aegis of a formal interagency agreement with that Institution. A project is underway in which acute stroke patients are admitted to the Neurology Intensive Care Unit and evaluated for evidence of clinical progression during the first 24 hr after admission. If, with close observation, the incidence of progression in this population is in the range of 30-40% (as suggested by the literature), early deterioration could be a focus for future acute interventional studies. In addition, proposals are being drafted for studies of perivascular monocyte and macrophage interaction with endothelium in stroke patients mediated by cytokines such as tumor necrosis factor -alpha (TNF- α) and interleukin-1 (IL-1).

Two lines of investigation are being pursued in the basic research program of the Clinical Investigation Section. The first is a more definitive approach to the hypothesis that perivascular macrophage interaction with endothelium mediated through TNF- α and IL-1 underlies the increased likelihood of stroke associated with stroke risk factors. Rats with hypertension or old age are compared with risk factor-free control rats. Expression of adhesion receptors such ICAM-1 and accumulation of perivascular monocytes and macrophages are compared among these groups of animals. In addition, the output of cytokines by stimulated carotid artery segments and brain microvessel endothelial cell adhesion receptor expression and binding of monocytes are also compared among the animal groups. The relevance of an exaggerated communication between perivascular macrophages and endothelial cells via cytokines to stroke will be assessed directly by blocking TNF- α and IL-1 in spontaneously hypertensive stroke-prone rats which will develop strokes between one and three months of age if they are unprotected.

The other major area of basic research in the Clinical Investigation Section involves developing an effective therapy for stroke. The self-destructive processes set into motion by stroke are extremely multifactorial, and to date have eluded definitive therapy. Multiple agent therapy would seem to be a rational approach, but complex and unpredictable interactions among the agents as well as other difficulties with this intervention have generally rendered such efforts disappointing. A novel strategy for modulating the intricate and interwoven destructive processes set into motion by stroke would be to seek a master signaling molecule which, in effect, would shut down cellular alarms and profoundly blunt the responses of cells exposed to hypoxia and ischemia. There are such states occurring in nature. An example is hibernation, in which mammalian brain cells tolerate heart rates of 7-10

beats per minute (normal 350 -400 beats per minute) and occasional breaths, a condition which would lead to rapid autolysis of normal brain. There is evidence that this state may be induced by a neuropeptide signal emanating from the hypothalamus. We plan to isolate and characterize such a neuropeptide in hibernating ground squirrels by standard protein purification methods and molecular genetic approaches. Also, the degree of neuronal protection conferred by hibernation will be assessed in models of brain injury.

The research effort of the Section on Cerebrovascular Pathophysiology has remained focussed on elucidating pathomechanisms in cardiac arrest-induced cerebral ischemia. Since this experimental model closely reflects pathophysiologic events in human cardiac arrest cases, our studies are directly relevant to designing proper measures for clinical management of this condition.

Ischemic brain damage following cardiac arrest represents a most serious and common complication, and reveals some pathologic features which differ from those found in other types of ischemia, characterized by transient or permanent occlusions of various brain arteries. These differences may be related to the totality of circulatory cessation in cardiac arrest affecting all organs of the body, which is not present in cerebral ischemia due to arterial occlusions with preserved heart activity. Two pathophysiologic features of cardiac arrest which may affect the character of ischemic injury in cardiac arrest are as follows. First, cardiac arrest produces a total flow interruption of both arterial and venous blood, as well as that of cerebrospinal and interstitial fluids. This prevents removal of any toxic or metabolic products accumulating during ischemia. Second, since there is total ischemia, production of toxic metabolites occurs in all organs, and after resuscitation, there is a good possibility that these products (circulating after resuscitation) may enter the brain due to increased permeability of the blood-brain barrier (BBB). The breakdown of the BBB shortly after resuscitation from cardiac arrest has been recently demonstrated by Gannushkina et al. (1990).

During the past year, using the cardiac arrest procedure by compressing major cardiac vessels, we have reported a striking, widespread involvement of GABAergic neuronal elements, becoming evident shortly following recirculation. Using GABA and glutamate decarboxylase (GAD) immunohistochemistry to assess GABA neurotransmitter levels, as well as parvalbumin (PV) immunostaining as a marker for GABAergic neurons, our observations revealed during postischemic periods different patterns of change among the GABAergic elements during postischemic periods. The first phase, manifest 1 hr after resuscitation, was characterized by enlargement and intense immunostaining of GABAergic terminals and boutons, best seen in the ventral thalamic nuclei (VTN), adjacent to the nucleus reticularis thalami (NRT). Immunostaining of the latter revealed loss of GAD and GABA immunoreactivity in about 80% of GABAergic neurons. The changes in immunoreactivity of GABAergic boutons at 1 hr were widespread and were particularly evident in the cerebral cortex, hippocampus and inferior colliculus. Application of GAD and GABA on the ultrastructural level revealed marked swelling of GABAergic boutons with dense staining of enlarged synaptic vesicles. The second phase of changes in GABAergic elements (1-7 days) was characterized by almost complete disintegration of GABAergic terminals in the VTN and moderate disappearance of GABAergic neurons in the inferior colliculus, whereas GABAergic elements in other locations showed full recovery. The third phase of changes (from day 7 after recirculation) was marked by regenerative sprouting and new formation of terminals and boutons in the VTN. Among other locations that showed changes in GABAergic neuronal elements at 1 hr, only the inferior colliculus revealed some degree of sprouting.

The sprouting and regeneration of GABAergic terminals was associated with high levels of growth-associated protein (GAP)-43 mRNA expression, demonstrated in the NRT by nonradioisotopic *in situ* hybridization. Rats sacrificed 7 days after recirculation selectively showed, in the NRT, an intense GAP-43 mRNA dark staining of scattered neurons and some globular material in the neuropil. No enhanced GAP-43 mRNA expression was observed in other locations or in rats sacrificed at other time intervals. The observations on sprouting of GABAergic terminals and on activation of GAP-43 mRNA constitute important new findings on regenerative phenomena following cerebral ischemia, and are of potential significance for pharmacologic manipulation of recovery of ischemically injured neurons.

The regenerative capacity of the neurons after ischemia was also suggested from our observations on the dynamics of postischemic *in vivo* calcium uptake and protein synthesis. In these studies, observations in the hippocampal CA1 sector indicated that a significant degree of protein synthesis, maintained at the late stage after postischemic recovery, was related to survival and regeneration of neurons and not to activity of glial elements. Otherwise, the scattered remaining CA1 neurons indicated an increased protein synthesis and regenerative tendency by the presence of distinct, single nucleoli and prominent Nissl substance, whereas pyramidal neurons in control animals showed mostly small multiple nucleoli.

One of the striking features in our model was development of susceptibility to audiogenic seizures, which could be evoked by the rattling of keys. Studies on the relationship of audiogenic seizures to morphologic changes revealed the following observations. The onset of susceptibility to audiogenic seizures occurred at 24 hr following cardiac arrest and coincided with the appearance of severe cellular injury to the somatostatin neurons in the hilus of the dentate gyrus of the hippocampus. The susceptibility to audiogenic seizures lasted for about one month, following which it subsided in most of the postischemic rats, and coincided in time with the period of most intense sprouting and formation of new GABAergic terminals and boutons. Lesioning of the inferior colliculus resulted in prevention of the audiogenic seizures, otherwise, cortical, hippocampal or thalamic lesioning had no evident effect on susceptibility to seizures. On the other hand, several animals subjected to small, shallow lesions in the parietal cortex and one week later exposed to cardiac arrest induced ischemia and sacrificed 7 days after cardiac arrest, showed a strikingly good preservation of CA1 and NRT neurons, which in control animals without cortical lesions regularly showed advanced neuronal destruction. Our current attempts are focussed on confirming this observation in a larger number of animals and by relating this neuronal protection to the spreading depression, which is likely to be induced by parietal cortex lesions.

The observations made last year, described above, influence in great measure the direction of our contemplated future studies. Most important appears to be a further pursuit of our findings on regenerative phenomena, since it may lead to measures which could be beneficial in treating postischemic brain damage. Our rationale is based on the assumption that an ischemic insult may be associated with large territories where the neuronal elements, although injured, try to recover and regain full function. Sprouting of new terminals derived from severely injured NRT neurons is an indication of such regenerative power, and it is possible that pharmacologic manipulation of this tendency may accelerate or facilitate a functional recovery in patients. A putative amelioration of ischemic neuronal injury by a distant, circumscribed cortical injury, produced 1 week preceding cardiac arrest injury, suggests a possibility that a cortical irritation, presumably associated with the

spreading depression, may induce a chain of events perhaps related to genetic expression and second messenger changes and which may provide protection from neuronal injury in various brain regions. Pharmacologic manipulation of this phenomenon could provide insight into mechanisms involved and may suggest new avenues for therapeutic intervention.

The continuing goals of the Section of Neurocytobiology have been to: (I) develop and utilize model systems *in vitro* for investigation of the basic mechanisms which might be operative *in vivo* on the level of the normal and pathologically altered endothelium and endothelial cell interactions; (II) to study the pathophysiologic mechanisms of ischemia which may be involved in brain injury but at the same time provide clues for single or multifactorial treatment; and (III) to evaluate the influence of genetic and immunologic factors on the generation of ischemia and autoimmune diseases involving the central nervous system (CNS).

I. During the past year, cultured endothelial cells (EC) derived from dissociated microvessels of human, rat and mouse brain have been constantly used as "living cell" models *in vitro* for the investigation of their interaction with leukocytes and/or substances (such as cytokines or peptides) derived from either brain or blood cells. These studies have focused on two main aspects of EC function: (a) receptor-mediated secretion, modulation and signal transduction mechanisms; and (b) expression and modulation of surface molecules. Both of these processes may take part in the development and/or progression of hypertension, vasospasm, stroke and autoimmune diseases of the CNS. Concurrently, a considerable effort has been made to develop and establish additional lines of EC cultures derived from brain microvessels of spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats. This successful venture has enabled us to join and collaborate in the studies initiated by Dr. John Hallenbeck, Chief of the Stroke Branch and Clinical Investigations Section (CIS) to elucidate changes in EC properties induced by cytokines which hypothetically predispose to the development of stroke (see above for Dr. Hallenbeck's hypothesis of stroke risk factors).

IA. Endothelins have been detected in the cerebrospinal fluid of patients with various cerebrovascular disorders and are implicated as predisposing factors for these diseases (posttraumatic vasospasm, hypertension, stroke, etc.). Endothelins are a group of 21- amino acid peptides secreted by EC and expressed as three isoforms in the human genome (endothelin-1, -2, -3). Endothelin 1 (ET-1) has potent (cerebro)vasoconstrictor activity both *in vitro* and *in vivo*. In addition to endothelium, the presence and/or secretion of immunoreactive ET-1 has been shown in vascular smooth muscle cells, various glial cells, and neurons. Many of the vascular reactivities under normal and pathologic conditions are mediated by specific receptors. Recently, we demonstrated a peptidergic (angiotensin II or vasopressin) receptor-mediated induction of ET-1 and prostanoid secretion which can be modulated by cyclooxygenase, lipoxygenase and phospholipase A₂ inhibitors by human brain microvascular endothelium (HBEC). Since cerebral microvascular function can also be influenced by ET-1 produced by smooth muscle or glial cells, we localized and characterized ET-1 receptors and investigated the effect of exogenous ET-1 or ET-3 on HBEC production of prostanoids which may contribute to the vascular response.

The presence of both high and low affinity binding sites for ET-1 was demonstrated on intact HBEC. ET-1 dose-dependently stimulated inositol phosphate (IP) accumulation, while ET-3 was ineffective. The order of potency for displacing ET-1 from high affinity binding sites (IC₅₀ was ET-1 > ET-2 > sarafotoxin S6b > ET-3) correlated exponentially with the ability of the respective ligands to induce IP₃ formation. The

protein kinase C (PKC) activator phorbol myristate ester (PMA) dose-dependently blocked the ET-1 stimulated production of IP_s, while pertussis toxin (Ptx) was ineffective. cAMP production by HBEC was enhanced by both PMA and ET-1, and synergistically potentiated by combined treatment with ET-1 and PMA. Data indicate that PKC plays a role in regulation of ET-1-induced activation of phospholipase C (PLC), while interaction of different messenger systems may regulate ET-1-induced accumulation of cAMP.

ET-1 also dose-dependently stimulated prostaglandins [thromboxane B₂ (TxB₂), prostaglandin F_{2α} (PGF_{2α}), 6-keto prostaglandin F_{1α} (6-keto PGF_{1α}), prostaglandin E₂ (PGE₂), and prostaglandin D₂ (PGD₂)] production by ET-1 in capillary EC derived from HBEC. Prostaglandin formation (with the exception of PGD₂) was blocked by both dexamethasone (50 μM) and neomycin (50 μM). The increase in the vasoconstrictive prostaglandin TxB₂ and PGF_{2α} temporally preceded that of the vasodilatory 6-keto PGF_{1α}, PGE₂, and PGD₂ and was already seen after 15 min of incubation with ET-1 (10 nM). Increased production of vasodilatory prostaglandins was observed between 4-8 hr of incubation, whereas normalization of both vasoconstrictive and vasodilatory prostaglandins occurred 24 hr after addition of ET-1. In contrast, ET-3 had no effect on prostanoid production by HBEC. ET-1 stimulated prostaglandin secretion by HBEC was diminished by verapamil (10 μM) suggesting that activation of phospho-lipase A₂ (PLA₂) by ET-1 was partly induced by extracellular calcium influx. ET-1 stimulated endothelial TxB₂ and PGF_{2α} production suggesting that activation of PLA₂ is most likely secondary to IP₃-type receptors linked to PLC and PLA₂ activation in HBEC.

The presence of ET-1 receptors and the temporal profile of various prostanoids induced by ET-1 in the EC strongly suggests an existing delicate balance between vasoconstrictive and vasodilatory endothelial factors which may affect the vascular tone. However, based on these results alone (without investigating capillary physiologic parameters), it is difficult to predict the final effect of ET-1 on the capillary bed, which can also be influenced by substances released from both surrounding brain cells (pericytes, smooth muscle, glial cells, neurons) and blood cells. Nevertheless, the detected ET-1 receptors and the response of the various prostanoids to ET-1 in EC support the working hypothesis that the endothelium can play a significant role in the response of capillaries (dilatation, constriction, permeability) under physiologic and pathologic conditions.

IB. The transvascular migration of lymphocytes, monocytes and other leukocytes from the blood to the CNS is characteristic of many CNS disorders such as stroke, multiple sclerosis (MS) and experimental allergic encephalomyelitis (EAE). The emigration of cells into peripheral tissues is preceded by adhesive interactions utilizing specific molecules (e.g., integrins) on the surface of leukocytes and EC. Such adhesive interactions could also lead to local vessel occlusion and circulatory impairment resulting in circumscribed ischemia or hemorrhagic tissue damage characteristic of disorders such as atherosclerosis and stroke. Our studies on both EC derived from mice (SJL) and rats (SHR and WKY) demonstrated the expression of intercellular adhesion molecules (ICAM-1). In addition to ICAM-1, other molecules were observed using monoclonal antibodies (mAb) prepared from rats inoculated with murine cerebrovascular EC. The results also showed that ICAM-1 expression by cerebrovascular EC can be modulated by cytokines (i.e., tumor necrosis factor-α [TNF-α]; interleukin-1 [IL-1]; interferon-γ [IFN]).

Investigation of the interaction between EC and peripheral blood leukocytes has shown that murine T lymphocytes capable of transferring EAE and peripheral blood monocytes from WKY and SHR rats adhered to syngeneic cerebrovascular EC.

Treatment of murine cultures with IFN, IL-1, and/or TNF- α up-regulated adhesion in a time- and dose-dependent manner. Pretreatment of EC with transforming growth factor- β (TGF- β) partially inhibited T cell adhesion to untreated EC and down-regulated the effects of the aforementioned cytokines on adhesion. EC treated with TGF- β down-regulated the level of T cell adhesion on untreated EC, and inhibited the up-regulation of adhesion induced by IFN, IL-1 and/or TNF. However, TGF- β had no effect on the ability of IFN, IL-1 and/or TNF to up-regulate ICAM expression. These results indicate that T cell adhesion and ICAM expression are not totally dependent phenomena.

Similarly, treatment of rat EC cultures with IFN, IL-1, TNF, LPS, PMA and A23187 up-regulated adhesion both time- and dose-dependently. The relative degree of enhancement was greater on SHR EC than WKY EC. The results indicate that these factors can regulate EC-leukocyte interactions which may result in permeability changes or conversion of endothelium to a procoagulant surface at the site of the BBB. Such changes may lead to local thrombosis/hemorrhage which is characteristic of a disorder such as stroke or they may affect peripheral immune cell egression into the CNS, which is a pathologic hallmark of neuroimmune disorders such as EAE and MS.

II. The continuous study of cerebral ischemia, its pathophysiology and therapy have been focused on investigations of: (a) regional dopamine (DA) turnover and free radical membrane disturbances induced by ischemia and reperfusion; and (b) elucidate the effect of ischemia on acetylcholine (ACh) metabolism in the gerbil model of transient global ischemia.

IIA. These investigations revealed that marked extracellular (observed by microdialysis) DA release, decrease of monoamine oxidase (MAO)-derived metabolites (3,4-dihydroxyphenylacetic acid and homovanillic acid), and accumulation of 3-methoxytyramine (3-MT) during ischemia and the first 15 min of reperfusion were greatly potentiated by pargyline pretreatment. Changes in the contents of DA and its metabolites, as well as in the increase in superoxide radical production, decrease of superoxide dismutase activity, and thiobarbituric acid-reactive material accumulation were more pronounced in the striatum than in the cortex. The ischemic release of DA is followed by a shift of DA metabolism toward production of 3-MT during reperfusion due to inhibition of MAO was confirmed by pargyline pretreatment. The observed imbalance between superoxide anion/ superoxide dismutase ratio and accumulation of thiobarbituric acid-reactive material support the contention that inhibition of DA clearance from the extracellular space during reperfusion may contribute to the increased free radical formation and membrane damage in the DA-rich regions such as the striatum.

The present study demonstrates for the first time that the temporal profile and regional degree in changes of DA metabolism correlate well with the apparent intensity of oxidative stress (superoxide radical production and *in vitro* lipid peroxidation) in the brain caused by ischemia/reperfusion. These and other investigations strongly support the notion that the greater susceptibility of the striatum to ischemia (as compared to the cortex) can be attributed to its intrinsic biochemical properties. Among other factors, the rich dopaminergic input, high content of inorganic ferrous complexes, and the inadequate capacity of the antioxidative system facilitate production and activity of free radicals which play a significant role in striatal ischemia damage.

IIB. The effect of cerebral ischemia on ACh metabolism was investigated in the same gerbil model of ischemia which has been used to study other metabolic pathways in order to examine the pathomechanism involved in ischemic tissue injury, and at the same time to provide clues for a possible single or multifactorial approach for prevention and/or therapy. Transient forebrain ischemia (15 min) induced an increase in extracellular ACh concentration, concomitant with a reduction in endogenous ACh levels and an increase in tissue choline content. Recirculation led to a significant reduction of the extracellular ACh concentration during the early phase of reflow, followed by a significant transient increase in the ACh release between 1 and 3 hr of reflow with subsequent normalization. In the meantime, a rebound of the tissue ACh levels was found during the early phase of reflow, followed by a gradual normalization after 2 hr of reperfusion, whereas the rapid decrease in tissue choline levels occurred after 30 min of reflow. These data represent the first report of a biphasic striatal ACh release occurring during transient ischemia and reperfusion, assessed by cerebral microdialysis in gerbils. These findings, in addition to the observed change in postischemic release of other neurotransmitters, altered 5-HT₁ and 5-HT₂ binding properties as well as fluidity of the membrane strongly suggest that the mere resupply of the blood to the brain after ischemia is not sufficient for recovery. These observations are important not only to unravel the pathomechanism of cerebral ischemia but should be taken into consideration in the design of therapeutic approaches for ischemia. An evaluation of a single and a multifactorial drug treatment is in progress in the same experimental model of ischemia.

III. Dr. McCarron in collaboration with Dr. J. Rose (VAMC, Salt Lake City, Utah) and Dr. F. Noonan (George Washington Univ. Med. Center, Washington, D.C.) examined the role of IL-2 receptor bearing cells in EAE using the chimeric protein IL2-PE40 which is cytotoxic for the above-mentioned cells. Early treatment of mice with IL2-PE40 (1-4 days post-transfer of encephalitogenic lymphocytes) prevented the expression of clinical signs of EAE. Administration of IL2-PE40 at the onset of clinical symptoms significantly reduced the severity of the disease and also prevented the subsequent development of relapses. This treatment also resulted in decreases in the level of demyelination and in the degree of inflammatory responses observed in the brain and spinal cord.

Immunization of SJL mice with myelin basic protein (MBP) resulted in the generation of MBP-specific T lymphocyte responses. After *in vitro* culture with MBP, these cells passively transferred EAE into naive recipients. UV irradiation of donor mice resulted in strong suppression (70-80%) of the immune responses, as measured by MBP-specific proliferative responses of immune T cells. It was also observed that UV irradiation of recipient mice (prior to passive transfer of MBP-specific T cells) suppressed both the incidence and severity of disease.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 N5 02856-01 SB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hibernation - A New Approach to Stroke Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. K. U. Frerichs, M.D.

Visiting Associate

SB/NINDS

Others:

J. M. Hallenbeck, M.D.

Section Chief

SB/NINDS

COOPERATING UNITS (if any)

L. Sokoloff, M.D., C. Kennedy, LCM.NIMH; J. Joy, C. Merrill, M.D., NIMH; H. Gainer, Ph.D., H. Jaffe, Ph.D., LNC/NINDS; M. Brenner, Ph.D., LMB/NINDS

LAB/BRANCH

Stroke Branch

SECTION

Clinical Investigation Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.9

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

No definite therapy for human stroke is currently available. Experimental stroke therapy so far has been based on the assumption that one, or at most, a few factors control the ischemic cell damage. There is a finite possibility that this assumption is wrong. Among the many factors participating in progression of neuronal cell loss, there may be none which are dominant. They may act instead as a network of minor causes, with intricate relationships among the various mediators and without clear sequence or directionality. The importance of considering this possibility is that the conventional search for a dominant mediator of ischemic cell death in stroke may be incompatible with the essential nature of the problem. As a new and alternative approach, we have started to investigate a state in nature, in which the tendency for cells exposed to cerebral ischemia to activate self-destructive processes, may be blunted, i.e., hibernation.

Thirteen-lined ground squirrels were used as the hibernating species. Hibernation under laboratory conditions in this species was characterized by remarkable adaptive changes, including severe hypothermia, rapid onset bradycardia and hypotension, all of which are rapidly reversible. During deep hibernation, cerebral blood flow (CBF) levels were below the ischemic threshold, but no neuropathologic damage could be detected. The tendency to activate self-destructive processes in hibernation under conditions of low CBF is markedly reduced. Possible mechanisms of protection include reversible changes in white blood cells and platelets, changes in cell membrane fragility and maintenance of ion gradient homeostasis. Furthermore, we have preliminary evidence that humoral factors of such regulatory factors may enable us to prevent the breakdown of homeostatic mechanisms during cerebral ischemia in other species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02865-01 SB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Stroke Risk Factors and Macrophages Endothelium Interplay		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	J. M. Hallenbeck, M.D.	Chief SB/NINDS
Others:	M. Spatz, M.D. R M McCarron, Ph.D. L Wang, M.D.	Section Chief Special Expert Visiting Fellow SB/NINDS SB/NINDS SB/NINDS
COOPERATING UNITS (if any) A-L Siren, L. Yong, Department of Neurology, Uniformed Services University of the Health Sciences, Bethesda, MD		
LAB/BRANCH Stroke Branch		
SECTION Clinical Investigation Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS	1.5	PROFESSIONAL: 1.5 OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unredused type. Do not exceed the space provided.) <p>The primary objective of the proposed work is to continue to investigate the following hypothesis: <u>Hypertension</u> and <u>advanced age</u>, the two major <u>risk factors</u> for <u>stroke</u> create a state in which the probability of an interaction between monocyte/macrophages and endothelial cells which could lead to local thrombosis or hemorrhage in focal regions of the brain vasculature is increased. Our preliminary data have demonstrated that animals with stroke-risk factors, when provoked by an appropriate stimulus, release more <u>tumor necrosis factor</u> (TNF-α) and undergo a more intense activation of hemostatic and proinflammatory mechanisms than risk factor-free controls probably as a result of a more vigorous interaction between their monocyte/macrophages and endothelium. The general plan for the next three years is to compare the activity and functional state of stimulated monocyte/macrophages and endothelium from animals with and without risk factors for stroke. The analyses will explore the possibility that several risk factors for stroke are initially associated with a change in endothelium.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02718-07 SB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Cerebral Electrical Activity Associated with Ischemia and Brain Injury

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H.G. Wagner, M.D.

Scientist Emeritus

SB/NINDS

Others: S. Xu, M.D.

Visiting Fellow

SB/NINDS

I. Klatzo, M.D.

Section Head

SB/NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Cerebrovascular Pathophysiology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.1

PROFESSIONAL:

2.1

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Early efforts to detect and analyze the effects of injury in single neurons of the cortex following transient ischemia or cold lesion were unsatisfactory. Some success in detecting change became evident in examining power spectra of the EEG. The postischemic EEG showed a shift of the dominant frequency from about 5HZ to 1-3 HZ and remained for more than 1 week. Visually evoked potentials clearly showed pronounced reduction in latency of 5-9 msec. Within 4-6 hours after placement of the cold lesion, the latency on the lesioned side began to decrease reaching 37 + 2 msec in 1-3 days. A similar reduction was observed in gerbils following transient ischemia by occlusion of the common carotid arteries. These shortened values were stable for more than the 2 hours that they were monitored. This reduction of latency in the visually evoked pathway is interpreted as evidence of hyperexcitability. Further support for this view was given by our finding that the reduced latency was quickly abolished by the administration of MK-801, and that a similar effect was produced by a direct application of glutamate to the exposed cerebral cortex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02720-06 SB
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Stress Protein Induction in Brain After Ischemia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	T.S. Nowak, Jr., Ph.D.	Special Expert SB, NINDS
Others:	S. Suga, M.D. N. Saito, M.D. K. Kawai, M.D.	Guest Researcher Visiting Fellow Visiting Fellow SB, NINDS SB, NINDS SB, NINDS
COOPERATING UNITS (if any) J.F. Mill, LMB, NINDS; M. Heyes, LCS, NIMH; S. Nadi, LNP, NINDS; P. Lindberg, USUHS; M. Jacewicz and W. Pulsinelli, Dept. Neurology, Cornell Univ. School of Med., New York		
LAB/BRANCH Stroke Branch		
SECTION Cerebrovascular Pathophysiology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	2.3	PROFESSIONAL: 2.3 OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Ongoing studies continue to focus on the role of <u>altered gene expression</u> in <u>postischemic pathophysiology</u>. Specifically, transcriptional and translational expression of the <u>stress protein, hsp70</u>, and <u>transcription factors, Fos and Jun</u>, are evaluated by comparison of <u>in situ hybridization</u> and <u>immunocytochemistry</u>. Perhaps the most significant result has been the observation that <u>threshold ischemic insults</u>, resulting in an <u>induced tolerance</u> to subsequent challenges, are correlated with hsp70 and Jun expression in the vulnerable CA1 neurons that are protected. Protein synthesis deficits that follow severe initial insults apparently result in the failure to translate mRNAs even though they may be induced. Since Jun in turn functions to regulate the transcriptional expression of other genes, this finding begins to indicate the complexity of genetic reprogramming that must be associated with induced tolerance phenomena.</p> <p>Other changes in gene expression have been evaluated after ischemia, notably that of <u>microtubule-associated protein 2 (MAP-2)</u>. An mRNA encoding a truncated protein, MAP-2c, is induced in cortex and hippocampal CA1 regions that show delayed damage in a rat cardiac arrest model. In contrast to stress protein and proto-oncogene expression that largely occur in surviving neurons, MAP-2c appears to constitute a <u>selective marker for severely injured neurons</u> after ischemia.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02773-04 SB
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Observations on Global Cerebral Ischemia in Rats		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	K. Kawai, M.D.	Visiting Fellow SB/NINDS
Others:	N. Saito, M.D.	Visiting Fellow SB/NINDS
	T.S. Nowak, Jr., Ph.D.	Special Expert SB/NINDS
	G. Mies, M.D.	Visiting Associate SB/NINDS
	I. Klatzo, M.D.	Section Head SB/NINDS
COOPERATING UNITS (if any)		
LAB/BRANCH Stroke Branch		
SECTION Cerebrovascular Pathophysiology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS- 2.1	PROFESSIONAL: 2.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>These studies revealed most early and widespread reaction of <u>GABAergic system</u> following <u>cardiac arrest cerebral ischemia</u>. The damage of reticularis thalami was moderate in the inferior colliculus, whereas GABAergic system following cardiac arrest cerebral ischemia. was not. The damage of GABAergic neurons was most pronounced in the nucleus reticularis thalami, moderate in the inferior colliculus, whereas GABAergic neurons in other locations showed recovery.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02821-035B

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dynamics of Postischemic Calcium Accumulation and Protein Synthesis in Brain Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. Mies, M.D.	Visiting Associate	SB/NINDS
Others:	T. S. Nowak Jr., M.D.	Special Expert	SB/NINDS
	K. Kawai, M.D.	Visiting Fellow	SB/NINDS
	N. Saito, M.D.	Visiting Fellow	SB/NINDS
	I. Klatzo, M.D.	Chief	SB/NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS

0.8

PROFESSIONAL:

0.8

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/>	(a) Human subjects	<input type="checkbox"/>	(b) Human tissues	<input checked="" type="checkbox"/>	(c) Neither
<input type="checkbox"/>	(a1) Minors				
<input type="checkbox"/>	(a2) Interviews				

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Dynamics of pathological changes in brain tissue following cardiac arrest induced ischemia were evaluated using double tracer autoradiography of regional in vivo ^{45}Ca uptake as respective indicator of ischemic and ^3H leucine protein synthesis as measure of metabolic cell integrity. Observations on abnormal calcium accumulation suggested that they were related to ^{45}Ca uptake by either injured but still living neurons and/or by reactive glial elements. ^{45}Ca autoradiography demonstrated a high sensitivity of the nucleus reticularis thalami, hippocampal CA1 pyramidal layer, inferior colliculus, ventral thalamic nucleus, caudate nucleus, and parietal cortex to ischemic neuron injury. Regional ^3H leucine incorporation revealed that an initially widespread inhibition of protein synthesis was followed by its considerable recovery. Observations in the hippocampal CA1 sector and in the ventral thalamic nucleus (VTN) suggested that a significant degree of protein synthesis, maintained at the late stage after postischemic recovery, was related to survival and regeneration of neurons and not to the presence of glial elements.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02822-03 SB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Glutamate Microdialysis During Repeated Ischemia and Cold Lesions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. Saito, M.D.	Visiting Fellow	SB/NINDS
Others:	T. S. Nowak Jr., Ph.D.	Special Expert	SB/NINDS
	I. Klatzo, M.D.	Section Head	SB/NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFFYEARS:

0.8

PROFESSIONAL:

0.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/>	(a) Human subjects	(b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/>	(a1) Minors		
<input type="checkbox"/>	(a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Release of the excitatory neurotransmitter amino acid, glutamate, has been suggested to play a role in ischemic neuronal injury and its contribution to the cumulative injury seen after repeated ischemic insults, was investigated in the gerbil. In vivo microdialysis demonstrated equivalent, transient glutamate release during each of three repeated carotid artery occlusion in continuously anesthetized animals. Parallel studies identified a striking temperature dependence of glutamate release in the physiological range. Recent work has demonstrated a significant postischemic hyperthermia upon release of anesthesia and occlusion in the gerbil model that appears to be an important factor in determining the severity of ischemic injury. Our results suggest that there may be an interaction between temperature and glutamate release after repeated occlusion that could contribute to their cumulative impact under conditions of lasting hyperthermia between ischemic insults.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02832-02SB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunochemical Observations on Neurotransmitter Changes in Global Cerebral Ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. Kawai, M.D.	Visiting Fellow	SB/NINDS
Others:	C. Ruetzler	Biologist	SB/NINDS
	L. Nitecka, M.D.	Visiting Scientist,	SB/NINDS
	J. Lohr	Biologist	SB/NINDS
	I. Klatzo, M.D.	Section Head	SB/NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFFYEARS:

1.4

PROFESSIONAL:

0.7

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The immunohistochemical observations on compounds related to GABA revealed a widespread reactivity of GABAergic system shortly after the ischemic insult. After 1 week, in relation to severe injury of the nucleus reticularis thalami (NRT), there was beginning of sprouting of new GABAergic terminals observed in the ventral thalamic nuclei (VTN) and it reached its peak at 30 days after ischemia. The sprouting represents a regenerative phenomenon which can be studied further by testing of various drugs.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02833-02 5B

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ear'y Neuronal Changes in Global Cerebral Ischemia

PRINCIPAL

PI:	K. Kawai, M.D.	Visiting Fellow	SB/NINDS
Others:	L. Nitecka, M.D.	Visiting Scientist	SB/NINDS
	N. Saito, M.D.	Visiting Fellow	SB/NINDS
	T.S. Nowak Jr., Ph.D.	Special Expert	SB/NINDS
	I. Klatzo, M.D.	Section Head	SB/NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS

1.6

PROFESSIONAL:

1.0

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies on global cerebral ischemia associated with cardiac arrest revealed striking neuronal changes in GABAergic neurons, as early as 15 min after recirculation. Although these changes may be transient, they may significantly influence the further development of ischemic injury. Further studies will be focussed on attempts to modify ischemic injury in global ischemia by pharmacologic means related to the GABAergic system.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02860-01 SB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Observations on Audiogenic Seizures in Rats Following Cardiac Arrest Cerebral Ischemia.

PRINCIPAL

PI:	K. Kawai, MD	Visiting Fellow	SB/NINDS
Others:	L. P. Penix, M.D.	Visiting Scientist	SB/NINDS
	C.A. Ruetzler	Biologist	SB/NINDS
	I. Klatzo, M.D.	Section Head	SB/NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS

1.6

PROFESSIONAL:

1.0

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several aspects of susceptibility to audiogenic seizures which develop in rats subjected to cardiac arrest cerebral ischemia have been investigated, revealing some interrelationships between epileptic activity and disinhibition due to dysfunction of certain GABAergic inhibitory systems. Further elucidation of these interrelationships is planned by pharmacological manipulations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 0057-15 SB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cerebral Ischemia and Neurotransmitters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. N. Bertrand, Ph.D. Visiting Fellow SB, NINDS

Others: H. Ishii, M.D. Visiting Fellow SB, NINDS
M. Spatz, M.D. Section Chief SB, NINDS

COOPERATING UNITS (if any)

Institute of Biochemistry, Faculty of Medicine, Belgrade, Yugoslavia (B. Mrsulja)

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.0 PROFESSIONAL: 0.9 OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The effect of cerebral ischemia on acetylcholine (ACh) metabolism was investigated in the same gerbil model of ischemia which has been used to study other metabolic pathways in order to examine the patho-mechanism involved in ischemic tissue injury, and at the same time to provide clues for a possible single or multifactorial approach for prevention and/or therapy. Transient forebrain ischemia (15 min) induced an increase in extracellular ACh concentration, concomitant with a reduction in endogenous ACh level and increase in tissue choline content. The recirculation lead to a significant reduction of the extra-cellular ACh concentration during the early time of reflow, followed by a significant transient increase in the ACh release between 1 and 3 hr of reflow with subsequent normalization. In the meantime, a rebound of the tissue ACh levels was found during the early time of reflow, followed by a gradual normalization after 2 hr of reperfusion, whereas the rapid decrease in tissue choline levels occurred after 30 min of reflow. These data represent the first report of a biphasic striatal ACh release occurring during transient ischemia and reperfusion, assessed by cerebral microdialysis in gerbils.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02623-08 SB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cerebral Ischemia and Edema: Biogenic Amines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: H. Ishii, M.D. Visiting Fellow SB, NINDS

Others: M. Spatz, M.D. Section Chief SB, NINDS
D. Stanimirovic, Ph.D. Visiting Fellow SB, NINDS

COOPERATING UNITS (if any)

Drs. Hideko and T. Yamamoto, Yokohama City University, Yokohama, Japan

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS 0.9

PROFESSIONAL: 0.9

OTHER: 0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study represents a continuous effort to elucidate ischemic pathophysiologic mechanisms which could be involved in the tissue damage but at the same time provide clues for a single or multifactorial approach to prevent and/or treat this disease. These investigations were concerned with studying the cerebral regional turnover of dopamine (DA) and free radical membrane disturbances in ischemia and postischemia. Marked DA release, decrease of monoamine oxidase-derived metabolites (3,4-dihydroxy-phenylacetic acid and homovanillic acid), and accumulation of 3-methoxytyramine during ischemia and the first 15 min of reperfusion were greatly potentiated by pargyline pretreatment. Changes in the contents of dopamine and its metabolites, as well as in the increase in superoxide radical production, decrease of superoxide dismutase activity, and thiobarbituric acid-reactive material accumulation were more pronounced in the striatum than in the cortex. The ischemic release of dopamine is followed by a shift of dopamine metabolism toward production of 3-methoxytyramine during reperfusion due to inhibition of monoamine oxidase was confirmed by pargyline pretreatment. The observed imbalance between superoxide anion/superoxide dismutase ratio and accumulation of thiobarbituric acid-reactive material support the contention that inhibition of dopamine clearance from the extracellular space during reperfusion may contribute to the increased free radical formation and membrane damage in DA-rich regions such as the striatum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 NS 02689-08 SB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Endothelin and Prostanoid Production in Cerebromicrovascular Endothelium

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. M. Spatz, M.D. Section Chief SB, NINDS

Others: D. Stanimirovic, M.D., Ph.D. Visiting Fellow SB, NINDS
 R.M. McCarron, Ph.D. Special Expert SB, NINDS

COOPERATING UNITS (if any)

S. Uematsu, M.D., Johns Hopkins Hospital, Baltimore, Maryland

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:

0.7

PROFESSIONAL:

0.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The vasoactive peptide, endothelin-1 (ET-1) has been implicated in the pathophysiology of various diseases that are accompanied by disturbances in the regulation of vascular tone. Recently, we have shown that human brain endothelial cells secrete both immunoreactive ET-1 and express high-affinity ET_A receptors coupled to activation of phospholipase C (PLC). The present study demonstrates a dose-dependent stimulation of prostaglandins [thromboxane B₂ (TxB₂), prostaglandin F_{2α} (PGF_{2α}), 6-keto prostaglandin F_{1α} (6-keto PGF_{1α}), prostaglandin E₂ (PGE₂), and prostaglandin D₂ (PGD₂) production by ET-1 in capillary endothelial cells derived from human brain (HBEC). Prostaglandin formation (with the exception of PGD₂) was blocked by both dexamethasone (50 μM) and neomycin (50 μM). The increase in the vasoconstrictive prostaglandins TxB₂ and PGF_{2α} temporally precede that of the vasodilatory 6-keto PGF_{1α}, PGE₂, and PGD₂ and was already seen after 15 min of incubation with ET-1 (10 nM). Increased production of vasodilatory prostaglandins was observed between 4-8 hr of incubation, whereas normalization of both vasoconstrictive and vasodilatory prostaglandins occurred 24 hr after addition of ET-1. In contrast, endothelin-3 (ET-3) had no effect on prostanoids production by HBEC. ET-1-stimulated prostaglandin secretion by HBEC was diminished by verapamil (10 μM) suggesting that activation of phospholipase A₂ (PLA₂) by ET-1 was partly induced by extracellular calcium influx. Data indicate that ET-1 activates PLA₂ both directly and indirectly through PLC activation, and subsequently induces formation of vasoactive prostanoids that might contribute to the vasoactive actions of ET-1 both qualitatively and quantitatively. The detected response of the various prostanoids to ET-1 in the HBEC strongly suggest that the endothelium can play a significant role in the reactivities of the capillaries (dilatation, constriction, permeability) under normal and pathologic conditions.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02777-04 SB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Cerebromicrovascular Endothelium: Studies *in vitro*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: M. Spatz, M.D. Section Chief SB, NINDS

Others: R.M. McCarron, Ph.D. Special Expert SB, NINDS

D.B. Stanimirovic, M.D., Ph.D. Visiting Fellow SB, NINDS

COOPERATING UNITS (if any)

Dr. S. Uematsu, The Johns Hopkins Hospital, Baltimore, MD

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.8

PROFESSIONAL:

0.5

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Capillary endothelial cells derived from human brain (HBCEC) synthesize prostaglandin D_2 (PGD_2) which can be stimulated, among other prostanoids, by endothelin1 (ET-1). Exogenous PGD_2 dose-dependently augmented the production of vasoconstrictive prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 9 α -11- β prostaglandin F_2 (9 α -11- β PGF_2), and thromboxane B_2 (TxB_2) and vasodilatory prostaglandin E_2 (PGE_2) as well as cAMP by HBCEC. These results suggest that PGD_2 may play a role in cerebral capillaries under physiologic and pathologic conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02795-04 SB
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Human Cerebromicrovascular Endothelial Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	D. Stanimirovic, Ph.D.	Visiting Fellow SB, NINDS
Others:	R.M. McCarron, Ph.D. M. Spatz, M.D.	Special Expert Section Chief SB, NINDS SB, NINDS
COOPERATING UNITS (if any) Dr. S. Uematsu, The Johns Hopkins Hospital, Baltimore, Maryland		
LAB/BRANCH Stroke Branch		
SECTION Section of Neurocytobiology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS	0.6	PROFESSIONAL: 0.3 OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The vasoactive <u>endothelins</u> have been detected in the cerebrospinal fluid of patients with various cerebrovascular disorders and implicated in predisposing vascular diseases (hypertension, vasospasm). Many of the vascular reactivities under normal and pathologic conditions are mediated by specific receptors. Therefore, the kinetic properties of <u>endothelin-1</u> (ET-1) binding sites and the production of <u>inositol phosphates</u> (IP₁, IP₂, IP₃), cAMP, thromboxane B₂ (TxB₂), and prostaglandin F_{2α} (PGF_{2α}) induced by various endothelins (ET-1, ET-2, ET-3, and sarafotoxin S6b) were examined in <u>endothelial cells derived from human brain microvessels</u> (HBEC). The presence of both high and low affinity binding sites for ET-1 with K_{D1} = 122 pM and K_{D2} = 31 nM and B_{max1} = 124 fmol/mg protein and B_{max2} = 909 fmol/mg protein, respectively, was demonstrated on intact HBEC. ET-1 dose-dependently stimulated inositol phosphate (IP) accumulation with EC₅₀ (IP₃) = 0.79 nM, while ET-3 was ineffective. The order of potency for displacing ET-1 from high affinity binding sites (IC₅₀ was ET-1 > ET-2 > sarafotoxin S6b > ET-3) correlated exponentially with the ability of the respective ligands to induce IP₃ formation. The protein kinase C (PKC) activator phorbol myristate ester (PMA) dose-dependently blocked the ET-1 stimulated production of IP₃, while pertussis toxin (Ptx) was ineffective. cAMP production by h.3EC was enhanced by both PMA and ET-1, and potentiated by combined treatment with ET-1 and PMA. Data indicate that PKC plays a role in regulation of ET-1-induced activation of phospholipase C (PLC), while interaction of different messenger systems may regulate ET-1-induced accumulation of cAMP. ET-1 stimulated endothelial TxB₂ and PGF_{2α} production, suggesting that activation of phospholipase A₂ (PLA₂) is most likely secondary to IP₃-mediated intracellular calcium mobilization. These findings are the first demonstration of ET-1 (ET_A-type) receptors linked to PLC and PLA₂ activation in HBEC. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02797-04 SB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cultures of Human Cerebromicrovascular Endothelium: Modulation of Endothelin Secretion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Maria Spatz, M.D. Section Chief SB, NINDS

Others: F. Bacic, M.D. Visiting Fellow SB, NINDS
R.M. McCarron, Ph.D. Special Expert SB, NINDS

COOPERATING UNITS (if any)

Dr. S. Uematsu, The Johns Hopkins Hospital, Baltimore, MD

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS 0.9 PROFESSIONAL: 0.5 OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Due to the limited availability of cultured human cerebromicrovascular endothelial cells, some aspects of these studies have been interrupted but others have been continued during the last fiscal year within project Z01 NS 02689-08 SB.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02324-16 SB

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Blood-Brain Barrier: In Vitro Model for the Study of Cerebrovascular Endothelial Permeability

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: R.M. McCarron, Ph.D.

Special Expert

SB, NINDS

Others: M. Spatz, M.D.

Section Chief

SB, NINDS

H. Ishii, MD

Visiting Fellow

SB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Under pathologic conditions such as ischemia or injury, the perturbation of endogenous scavenger enzymes (such as SOD, catalase, etc.) and the overproduction of oxygen radicals may contribute to central nervous system (CNS) injury. The experiments performed here are based on the hypothesis that the pathologic role of oxygen radicals in CNS ischemia and injury is related to alterations in endothelium membrane integrity. Oxygen radical formation by enzymatic as well as non-enzymatic generating systems is known to occur not only in endothelium, but in other brain cells. The experiments reported here demonstrate that exposure of endothelial cells (EC) to free radicals generated by EC incubated in the presence of hypoxanthine (or xanthine) and xanthine oxidase or glucose and glucose oxidase caused alterations in EC permeability, with accompanying changes in cytoskeletal actin filaments. The above effects of endothelium-derived free radicals were both time- and dose-dependent and could be inhibited by treatment with antioxidants. The findings indicate that formation of oxygen metabolites (free radical species) can lead to EC injury and may represent a potential mechanism responsible for alterations in the blood-brain barrier permeability which is known to occur in various neuropathologic disorders, including stroke and multiple sclerosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02776-04 SB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanism of Production of Experimental Allergic Encephalomyelitis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: R.M. McCarron, Ph.D. Special Expert SB, NINDS		
COOPERATING UNITS (if any) Dr. J. Rose, Neurovirology Res. Lab., VAMC, Salt Lake City, UT Dr. Frances Noonan, Dept. Dermatol., George Washington Univ. Med. Ctr., Wash., D.C.		
LAB/BRANCH Stroke Branch		
SECTION Section of Neurocytobiology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Experiments examining the role of IL-2 receptor bearing cells in <u>experimental allergic encephalomyelitis</u> (EAE) were performed using the chimeric protein IL2-PE40 which is cytotoxic for the above-mentioned cells. Early treatment of mice with IL2-PE40 (1-4 days post-transfer of encephalitogenic lymphocytes) prevented the expression of clinical signs of EAE. Administration of IL2-PE40 at the onset of clinical symptoms significantly reduced the severity of disease and also prevented the subsequent development of relapses. This treatment also resulted in decreases in the level of demyelination and in the degree of inflammatory responses observed in the brain and spinal cord. Immunization of SJL mice donors with <u>myelin basic protein</u> (MBP) resulted in the generation of MBP-specific T lymphocyte responses. After <i>in vitro</i> culture with MBP, these cells passively transferred EAE into naive recipients. UV irradiation of donor mice resulted in strong suppression (70-80%) of the immune response, as measured by MBP-specific proliferative responses of immune T cells. It was also observed that UV irradiation of recipient mice (prior to passive transfer of MBP-specific T cells) suppressed both the incidence and severity of disease.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02780-04 SB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cerebral Vascular Endothelial Cell-Specific Monoclonal Antibodies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: R.M. McCarron, Ph.D. Special Expert SB/NINDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Stroke Branch		
SECTION Section of Neurocytobiology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="min-height: 150px; vertical-align: top; padding-top: 10px;"> This project was subsumed within project number ZO1 NS 02802-04 SB </div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02801-04 SB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Interactions Between Cerebrovascular Endothelial Cells and Immune Leukocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	R. M. McCarron, Ph.D.	Special Expert SB/NINDS
Others:	J. Hallenbeck, M.D. M. Spatz, M.D.	Branch Chief SB/NINDS Section Chief SB/NINDS
COOPERATING UNITS (if any) Dr. D. McFarlin, NIB, NINDS; Dr. M. Racke, NIB, NINDS Dr. A.-L. Siren, Dept. Neurol., USUHS, Bethesda, MD		
LAB/BRANCH Stroke Branch		
SECTION Section of Neurocytobiology		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF-YEARS:	1.2	PROFESSIONAL: 1.2 OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The experiments described here were designed to study interactions which occur between <u>cerebrovascular endothelial cells</u> (EC) which comprise the <u>blood-brain barrier</u> (BBB) and peripheral blood leukocytes. It was observed that murine T lymphocytes capable of transferring experimental allergic encephalomyelitis (EAE) adhered to monolayers of murine cerebrovascular EC. Treatment of murine EC cultures with interferon-γ (IFN), interleukin-1 (IL-1) and/or tumor necrosis factor-α (TNF) up-regulated adhesion in a time- and dose-dependent manner. Pretreatment of EC with transforming growth factor-β (TGF) partially inhibited T cell adhesion to untreated EC and down-regulated the effects of the aforementioned cytokines on adhesion. It was also observed that peripheral blood monocytes from normotensive (WKY) and spontaneously hypertensive (SHR) rats adhered to syngeneic cerebrovascular EC. Treatment of rat EC cultures with IFN, IL-1, TNF, LPS, PMA and A23187 up-regulated adhesion in a time- and dose-dependent manner. The relative degree of enhancement was greater on SHR EC than WKY EC. The results indicate that these cytokines/factors can regulate EC-leukocyte interactions which may result in permeability changes or conversion of endothelium to a procoagulant surface at the site of the BBB. Such changes may lead to local thrombosis/hemorrhage which is characteristic of disorders such as atherosclerosis or stroke or they may affect peripheral immune cell egression into the CNS, which is a pathologic hallmark of neuroimmune disorders such as EAE and multiple sclerosis (MS).</p> <p>Murine cerebrovascular EC were also shown to synthesize and release prostacyclin (PGI₂) upon treatment with the cytokine IL-1. The ability of peripheral immune macrophages (MO) to release IL-1, the consequent stimulation of EC-derived PGI₂, as well as the inhibition of antigen (MBP)-specific proliferation of encephalitogenic T cells by PGI₂ were characterized.</p>		
-27-SB/DIR		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02802-04 SB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immune Mechanisms: Regulation of EC Surface Antigen Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: R.M. McCarron, Ph.D. Special Expert SB/NINDS

Others: J. Hallenbeck, M.D. Branch Chief SB,NINDS
 M. Spatz, M.D. Section Chief SB/NINDS
 L. Wang, M.D. Guest Researcher SB/NINDS

COOPERATING UNITS (if any)

M. Tanaka, Brain Research Institute, Niagata University, Niagata City, Japan
 A.-L. Siren, Dept. Neurol., USUHS, Bethesda, MD

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The transvascular migration of lymphocytes and other leukocytes from blood to the central nervous system (CNS) is characteristic of many CNS disorders such as stroke, multiple sclerosis (MS) and experimental allergic encephalomyelitis (EAE). The emigration of cells into peripheral tissues is preceded by adhesive interactions utilizing specific molecules (e.g., integrins) on the surface of leukocytes and endothelial cells (EC). Such adhesive interactions could also lead to local vessel occlusion and circulatory impairment resulting in circumscribed ischemia or hemorrhagic tissue damage characteristic of disorders such as atherosclerosis and stroke. These experiments demonstrate the expression of intercellular adhesion molecule-1 (ICAM-1) on cerebrovascular EC which constitute the blood-brain barrier (BBB). In addition to ICAM-1, other molecules were observed using monoclonal antibodies (mAb) prepared from rats inoculated with cerebrovascular EC. The results also show that ICAM expression by cerebrovascular EC can be modulated by cytokines (i.e., tumor necrosis factor- α , [TNF]; interleukin-1, [IL-1]; interferon- γ , [IFN]; and transforming growth factor- β , [TGF] which have clinical relevance to all the above-mentioned disorders. The results indicate that adhesion molecule expression by EC at the site of the BBB may play a pathologic role (i.e., leukocyte adhesion and/or transmigration) in a wide range of CNS disorders including stroke, MS and EAE.

ANNUAL REPORT

October 1, 1991 through September 30, 1992

Neuroepidemiology Branch
Clinical Neurosciences Program, DIR
National Institute of Neurological Disorders and Stroke

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**Annual Report
October 1, 1991 through September 30, 1992**

**Neuroepidemiology Branch
Division of Intramural Research
Clinical Neurosciences Program
National Institute of Neurological Disorders and Stroke
Gustavo C. Roman, M.D. Chief**

Introduction

The Neuroepidemiology Branch (NEB) is responsible for the development and implementation of studies of classic and clinical epidemiology to investigate the cause, prevention, and treatment of neurologic disorders in human populations. Neuroepidemiology has become an independent scientific and research discipline which has already contributed a wealth of information regarding the magnitude of neurologic disorders, risk factors, and possible pathogenesis and causes of these disorders. Neuroepidemiology is also a tool to generate etiologic hypotheses for the study of diseases of unknown etiology.

Organization of the Branch

NEB currently has only two senior scientists: Dr. Gustavo C. Roman, Chief, and Dr. Karin B. Nelson. During this fiscal year, Dr. Roswell Eldridge retired from the National Institutes of Health. The following fellows are currently serving in the Neuroepidemiology Branch: Dr. Aurora K. Pajeau, graduate from the University of Vermont; Dr. Irene Litvan, graduate from Georgetown University; Dr. Joseph M. Scheller, Pediatric Neurologist from the University of California San Diego; Dr. Joana Rosario from the University of Lisbon; Dr. Danyang Chen, from Beijing Union Medical College, Chinese Academy of Medical Sciences. Currently, Dr. Zhen Xin Zhang, from Beijing University Medical College, is working as a Visiting Scientist in the Branch. Dr. David Hart, from the University of Maryland, and Dr. Nimal Senanayake from the University of Peradeniya, Sri Lanka, completed their appointments as Fellow and Visiting Scientist, respectively, at the NEB.

Educational Activities

Despite the importance of neuroepidemiologic methods for practice and research, the number of neurologists with expertise in neuroepidemiology is quite limited. For this reason, the Neuroepidemiology Branch has continued a program of educational activities to inform young neurologists on the methods and goals of neuroepidemiology. The NEB participated in the teaching activities of the American Academy of Neurology. At the annual meeting in San Diego, California, in 1992, the NEB organized an half-day course entitled "Tools for Practice and Research: Understanding Neuroepidemiology," which received excellent reviews. The NEB has been asked to expand this activity to a full-day course. From the International viewpoint, the NEB, with support from the Fogarty International Center (FIC) organized the first "Latin American Course on Neuroepidemiology," which took place in Buenos Aires, Argentina, in October, 1991. A symposium in India was held on November, 1991, at the Bombay Hospital Institute of Medical Sciences, also with support from the World Federation of Neurology Research Groups on Tropical Neurology and Neuroepidemiology. Dr. Roman continues to serve as Chairman of the World Federation of Neurology Research Group in Neuroepidemiology, and Chairman of the WFN Annual Meetings. The last one took place on May 4, 1992 in

San Diego, California, the abstracts have been published in *Neuroepidemiology* (1992;11:102-110). Dr. Roman also organized the Vth Panamerican Symposium of Neuroepidemiology in Montevideo, Uruguay, in October, 1991. These activities have strengthened the opportunities for international research studies in the field of neuroepidemiology and have increased the interest of young neurologists in neuroepidemiology.

Research Activities

The Neuroepidemiology Branch continues to focus on the following main fields of research:

- 1) Retroviral diseases of the nervous system
- 2) Epidemiology of dementia and other neurodegenerative disorders
- 3) Pediatric neuroepidemiology
- 4) Tropical and geographical neuroepidemiology

Retroviral Disease of the Nervous System

HTLV-I Infections of the Nervous System

The first human retrovirus, HTLV-I, is considered the causal agent of tropical spastic paraparesis (TSP) and HTLV-I-associated myelopathy (HAM), as well as some forms of polymyositis and pseudo-amyotrophic lateral sclerosis. The NEB continues to work on implementation of the "Registry of HTLV-I Infections of the Nervous System," in order to obtain data on the magnitude of this problem in the Americas. In collaboration with the Viral Epidemiology Section, NCI, NIH, the NEB is also in the process of implementing a series of case-finding studies for TSP in Jamaica. During the last year, Dr. David Hart performed an intensive search for HTLV-I-associated myelopathy cases in Asuncion, Paraguay. Research on mechanisms of transmission of HTLV-I and evaluation of the role of the environment in migrants will be conducted in Brazil.

Epidemiology of Dementia and Other Neurodegenerative Disorders

Vascular Dementia

Dementia due to cerebrovascular disease is the second-most common cause of dementia in the elderly. However, due to the lack of diagnostic criteria, it has been difficult to compare international data. For this reason, the Neuroepidemiology Branch organized an International Workshop on Vascular Dementia held at the NIH, Bethesda, Maryland, in April, 1991. The diagnostic criteria for research studies on vascular dementia resulting from this meeting are scheduled for publication in *Neurology*. The Neuroepidemiology Branch is collaborating with the University of Pennsylvania on the use of the photon migration test to study perfusion of white matter in patients with Binswanger disease. Studies will also be conducted in association with the University of Massachusetts in the clinical and neuropsychologic characterization of vascular dementia in comparison with Alzheimer's disease.

Dr. Zhang continues to review the data on parkinsonism dementia-ALS in Guam. An extensive review of the literature on epidemiologic studies of Parkinson's disease has also been completed.

Pediatric Neuroepidemiology

In collaboration with the California Birth Defects Monitoring Program, NEB staff have designed and developed the California Cerebral Palsy Project. This project has established a population-based registry of children with cerebral palsy (CP) in four San Francisco Bay Area counties, for births 1983 through 1985. The purpose of this registry is to examine the prevalence and etiology of moderate or severe congenital CP, a handicapping and expensive form of chronic neurologic disability. [In 1988, costs to the state of California for diagnostic services, excluding physical and occupational therapy, which are themselves expensive, was nearly \$200 million; in addition there are costs to individuals and families.] A paper on the methodology and initial prevalence figures has been published as has one on dental observations on children with CP, intended eventually to investigate the use of dental markers as indicators of onset of maldevelopment. A paper on the demographic distribution of CP has been submitted, noting that women over age 35 have a higher risk of producing a child with CP, especially if they were also high in parity; that children of teenaged mothers or fathers were at somewhat higher risk. Infants born weighing less than 1000 g, who were 0.17% of survivors and a group who seldom lived past the neonatal period in the past, now contributed 8.7% of CP. Twins, 1.9% of the population, contributed 10% of the CP. A paper on twinning is now in preparation that further identifies factors that influence the risk for CP in twins. This is an increasingly important topic since twinning and higher-order multiple births are increasing markedly in the population due chiefly to fertility-enhancing medical therapies. Other analyses in this unique new data base are in preparation. Contact with families of affected children has begun in order to assemble and expand information on neuroimaging in these children as a component in the understanding of etiology of their disabilities. With coworkers at the Department of Neurology of the Children's National Medical Center, we are examining the reliability and sources of any inconsistency in the diagnosis of a first seizure in children. Studies of natural history of seizure disorders, and results of treatment trial of seizure disorders in children assume the reliability of diagnosis of seizures and determinations as to whether seizures are the initial such event, whether they are provoked by underlying illness, and their classification. We are testing the consistency of these diagnoses. Investigation of whether these terms are reliably employed, and endeavors to identify and resolve areas of inconsistency, are viewed as a mandatory step before better trials of medical treatment for seizures in children can be pursued with confidence.

NEB staff are involved in cooperative research with the Biometry and Field Studies Branch (BFSB) on the electroencephalogram as a predictor of recurrence in febrile seizures, and on several other aspects of risk factors for neurologic morbidity in children.

On April 2, 1992, the Neuroepidemiology Branch hosted a meeting of the Task Force of the World Federation of Neurology Group for the prevention of CP and related neurologic disorders, with support from the Little Foundation. The meeting discussed issues related to the appropriate use of the term "birth asphyxia" and on the importance of future research on neonatal encephalopathy.

Tropical and Geographic Neurology

Epilepsy

The Neuroepidemiology Branch continues to be interested in the high prevalence of epilepsy in tropical countries. Possibly, neurocysticercosis remains the main cause of

this problem. To study this disease, the Neuroepidemiology Branch has been collaborating with the Mexican Institute of Neurology and Neurosurgery in Mexico City. We have also studied the prevalence of neurocysticercosis in an asymptomatic population in endemic regions of Ecuador and Mexico.

Guillain-Barré Syndrome

The Guillain-Barré syndrome (GBS) has been reported with unusual frequency in the People's Republic of China. In collaboration with Beijing University Medical College, the Neuroepidemiology Branch will implement a surveillance mechanism for cases of GBS in China. In collaboration with the Panamerican Health Organization (PAHO), the NEB is also studying the reported cases of GBS in Latin America, as part of the program surveillance of poliomyelitis in Central and South America. A joint meeting of PAHO and NEB, NINDS, is scheduled for September 19, 1992, on "GBS and Acute Flaccid Paralysis in Childhood."

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 01924-22
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical, Genetic, Pathophysiologic Study of Hereditary Movement Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	Roswell Eldridge, M.D.	Medical Geneticist NEB, DIR, NINDS
Other	Robert Cohen, M.D. Elizabeth Mathew, M.D.	Chief LBI, IRP, NIMH Neurologist LBI, IRP, NIMH
COOPERATING UNITS (if any) CNB, DIR, NINDS; LCS, DCBR, NIMH; Department of Neurology, University of Mississippi School of Medicine, Jackson, Mississippi		
LAB/BRANCH Neuroepidemiology Branch		
SECTION		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	0.35	PROFESSIONAL: 0.3 OTHER: 0.05
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>In this project, we seek to 1) clarify and expand the nosology of the hereditary movement disorders; 2) contribute to the understanding of the underlying biochemical basis; 3) determine the most effective treatment and predictive testing for each disorder; and 4) suggest guidelines for counseling individuals at risk. General syndromes under study include the <u>dystonias</u>, <u>tic disorders</u>, the <u>ataxias</u>, and <u>myoclonus</u>. Approaches include standard <u>epidemiologic</u> and clinical and molecular <u>genetics</u> studies, and collaborative efforts in evaluating the role of <u>neurotransmitters</u> such as dopamine, and <u>PET studies</u>.</p> <p>We performed PET studies of at-risk members of two large kindreds with hereditary ataxia. Preliminary results suggested: a more generalized involvement than previously recognized; presymptomatic changes by PET; and evidence for genetic heterogeneity.</p> <p>This project has been completed.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z02 NS 01927-22 NEB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical, Genetic, Pathophysiologic Study of Hereditary Nervous System Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Roswell Eldridge, M.D.	Medical Geneticist	NEB, DIR, NINDS
Others:	Murial Kaiser-Kupfer, M.D.	Chief	OGB, IP, NEI
	Anita Pikus, M.S.	Audiologist	CA, IP, NIDCD
	Wesley McBride, M.D.	Molecular Geneticist	LBC, IP, NCI
	Judith Schaeffer, Ph.D./J.D.	Vestibulographer	CA, IP, NIDCD

COOPERATING UNITS (if any)

Division of Medical Genetics, Dept. of Pediatrics, Children's Hospital National Medical Center; Dept. of Neurosurgery, Massachusetts General Hospital, Boston, MA; Uniformed Services University of the Health Sciences, Beth, MD.

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	3.5	PROFESSIONAL:	3.0	OTHER:	0.5
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CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input checked="" type="checkbox"/> (a1) Minors		
<input checked="" type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was done to define and classify hereditary tumors of the nervous system such as occur in neurofibromatosis; to add to the clinical description and natural history of these diseases; to suggest methods for early diagnosis; to evaluate present modes of treatment; and to develop methods for preclinical detection and screening.

Our studies have led to the recognition of a preventable cause of deafness, visual loss or even death: neurofibromatosis 2 or bilateral acoustic neurofibromatosis. The genes for two distinct forms of neurofibromatosis have now been mapped to specific chromosomes. Recent contributions based on experience with over 100 individuals have been made at the clinical, genetic, epidemiologic, and management level. We are also organizing a consensus conference dealing with acoustic neuroma at which time the implications of these contributions will be weighed.

Our first major study involving neurofibromatosis 1 (NF1) a multidisciplinary project, demonstrated mild but consistent impairment of neurologic and cognitive status in these patients compared to their unaffected sibs. A second study assessing the burden of NF1 and attitudes towards predictive testing has been completed. There is great interest in such testing but most would not terminate a positive pregnancy.

This project has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02167-18 NEB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic Epidemiology Studies in Multiple Sclerosis and Other Multifactorial Neurologic Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.: Roswell Eldridge, M.D.	Medical Geneticist	NEB, DIR, NINDS
Others: Henry F. McFarland, M.D.	Assistant Chief	NI, DIR, NINDS
Walter A. Rocca, M.D.	Epidemiologist	NEB, DIR, NINDS
Gustavo C. Roman, M.D.	Chief	NEB, DIR, NINDS
Marinos Dalakas, M.D.	Neurologist	N. DIR, NINDS
Linda Nee, MSW	Research Associate	CNB, DIR, NINDS
COOPERATING UNITS (if any) BFSB, NEB, NI, CNB, NINDS; M; National Rehabilitation Hospital DC, Italian Multicenter Study on Dementia, SMID Centers, Florence, Italy; Departments Pediatrics and Neurology, King Faisal specialty Hospital, Riyadh, Saudi Arabia		
LAB/BRANCH Neuroepidemiology Branch		
SECTION		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.2	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>In this project we coupled genetic and environmental studies in selected families, twin pairs and populations with disorders such as <u>multiple sclerosis (MS)</u>, <u>Parkinson's disease (PD)</u>, and <u>Alzheimer's disease (AD)</u>, in an effort to distinguish specific contributing factors. Multidisciplinary twin studies of PD, MS, and AD indicate each is complex and involves an interaction of environmental and genetic factors.</p> <p>A study similar in design involving twins with documented polio over 30 years ago is designed to look at factors contributing to <u>'post polio' syndrome</u>.</p> <p>An autosomal dominant, <u>hereditary leukoencephalopathy</u> simulating MS with onset at about age 35 is under study in a kindred with over 20 affected. Derangement of the autonomic nervous system is often seen early in the course and when recognized clinically, serves to distinguish this single gene disorder from MS.</p> <p>In Saudi Arabia, a multidisciplinary study of an extended kindred with an autosomal recessive form of muscular dystrophy is planned. Epidemiologists and geneticists working together will assess the relative burden of environmental and genetic factors in this highly inbred population from the western part of Saudi Arabia.</p> <p>This project has been completed.</p>		
7 - NEB/DIR		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02240-16 NEB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidemiology of Dementia and Other Neurodegenerative disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Gustavo C. Roman, M.D.

Chief

NEB, DIR, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.05

PROFESSIONAL: 0.05

OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Analytic studies to determine risk factors for vascular dementia (VAD) and Alzheimer's disease (AD) are planned or being conducted. International studies on the prevalence and incidence of dementia and Parkinson's disease are planned in Argentina, Brazil, Colombia, and Panama. Risk factors associated with local conditions will be determined.

These studies are in the planning stages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02243-16 NEB

PERIOD COVERED

October 1, 1991 through September 30 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pediatric Neuroepidemiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS
Others:	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINDS
	Sherrie Emoto, Ph.D.	Staff Fellow	BFSB, DIR, NINDS
	Deborah Hirtz, M.D.	Medical Officer	DNB, NINDS

COOPERATING UNITS (if any)

Peter Scheidt, M.D., Medical Officer, NICHD

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	0.4	PROFESSIONAL:	0.3	OTHER:	0.1
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CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several studies on pediatric neuroepidemiology have reached the following stages: 1) completed last year; 2) the prenatal and perinatal antecedents of febrile seizures were examined in NINDS data; 3) with Yugoslav colleagues, we are examining the utility of the electroencephalogram as a predictor of recurrence of febrile seizures in a defined population in Yugoslavia; 4) 40 NINDS participants were involved in the analysis of a randomized controlled clinical trial undertaken in the NICHD to evaluate the safety and efficacy of phototherapy in the prevention/reduction of hyperbilirubinemia in the neonate. We investigated the frequency of adverse neurologic outcomes at six years of age.

Study 1 was completed last year. One manuscript published.
 Study 2 was completed. One manuscript published.
 Study 3 is now subsumed within project number Z01 NS 02715-07 NEB.
 Study 4 was completed. Two manuscripts published.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02307-16 NEB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Educational Resources in Neurological Epidemiology		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	Gustavo C. Roman, M.D.	Chief NEB, DIR, NINDS
Other:	Karin B. Nelson, M.D. Dallas W. Anderson, Ph.D.	Medical Officer Mathematical Statistician NEB, DIR, NINDS BFSB, DIR, NINDS
COOPERATING UNITS (if any)		
LAB/BRANCH Neuroepidemiology Branch		
SECTION		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	0.05	PROFESSIONAL: 0.05 OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Because there is a severe shortage of available manpower in <u>neuroepidemiology</u>, the Branch has developed an active <u>teaching program</u> for current and future collaborative investigators. Particular attention has been given to Junior Members of the American Academy of Neurology (Neurology residents). The NEB has participated actively in the Annual Courses of the American Academy of Neurology, in an effort to increase the interest in neuroepidemiology. To facilitate international research studies, educational activities have also been conducted in other countries.</p> <p>The following are some of these activities:</p> <p>Half-day neuroepidemiology course, American Academy of Neurology: "Tools for Practice and Research: Understanding Neuroepidemiology," San Diego, California. International Neuroepidemiology Course. Buenos Aires, Argentina, Fifth Pan American Symposium on Neuroepidemiology. Montevideo, Uruguay. Neuroepidemiology Symposium in Bombay, India. World Federation of Neurology, Research Group on Neuroepidemiology Annual Meeting, San Diego, California.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02370-14

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Racial and Geographic Differences in Occurrence of Neurologic Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Gustavo C. Roman, M.D.

Chief

NEB, DIR, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.05

PROFESSIONAL:

0.05

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of these studies is to accurately document possible racial, environmental and geographic differentials in the prevalence of major neurologic disorders by surveying an entire geographically defined population. The disorders investigated included cerebral palsy, dementia, psychomotor delay, epilepsy, Parkinson's disease, essential tremor, multiple sclerosis, and cerebrovascular disease.

Studies in Brasil and Argentina are in the planning stages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02715-07 NEB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Epilepsy Neuroepidemiology		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	Karin B. Nelson, M.D.	Medical Officer NEB, Dir, NINDS
Others:	Jonas H. Ellenberg, Ph.D. William Theodore, M.D. Sherrie Emoto, Ph.D.	Chief Medical Officer Staff Fellow BFSB, DIR, NINDS MNB, DIR, NINDS BFSB, DIR, NINDS
COOPERATING UNITS (if any) Judith Manelis, M.D., Western Galilee Regional Hospital, Israel		
LAB/BRANCH Neuroepidemiology Branch		
SECTION		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	0.8	PROFESSIONAL: 0.3 OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Several studies on convulsive disorders are being planned and tested for feasibility, or are in progress. A protocol is in effect for a clinical study of the <u>Lennox-Gastaut syndrome</u>(LGS), a severe childhood epileptic encephalopathy with significant morbidity, characterized by uncontrolled seizures, mental retardation, and possible mental deterioration, to define the pathophysiology and anatomic locus of disturbance in LGS. We are evaluating the feasibility of performing randomized and <u>placebo-controlled clinical trials</u> of treatment after an <u>initial convulsion</u> in subjects presenting for care to a consortium of hospitals in Jerusalem.</p> <p>With Yugoslav colleagues, we are examining the utility of the electroencephalogram as a predictor of recurrence of <u>febrile seizures</u> in a defined population in Yugoslavia.</p> <p>The clinical study of LGS is awaiting patient recruitment.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02746-06 NEB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phenobarbital Clinical Trial in Children with Febrile Seizures*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS
Others:	Deborah Hirtz, M.D.	Pediatric Neurologist	DNB, DCDND, NINDS
	Young Jack Lee, Ph.D.	Mathematical Statistician	NEB, DIR, NINDS
	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINDS

COOPERATING UNITS (if any)

Jacqueline Farewell, M.D., Dept. of Neurosurgery, Univ. of Washington, Seattle, WA

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	0.1	PROFESSIONAL:	0.1	OTHER:	0.0
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CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of the study are to assess the effects of phenobarbital, a commonly prescribed anticonvulsant, on tests of intelligence and behavior in children. The design of this study permitted comparison of measures of tested intelligence and of behavior in children with febrile seizures who had been treated with phenobarbital, and in a group of seizure-free control children. A comparison of the groups allowed assessment of benefit and risk of treatment for a common childhood neurologic problem.

*[This study supports the DNB/ND/NINDS contract study entitled: "Behavioral and cognitive side effects of phenobarbital used for prevention of febrile seizure recurrence." The project officer is Dr. Deborah G. Hirtz, DNB, DCDND, NINDS, and the contractor of the study is the University of Washington.]

Two papers submitted and additional data await analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02747-06 NEB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dental Markers of Maldevelopment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D.

Medical Officer

NEB, DIR, NINDS

COOPERATING UNITS (if any)

Mohandas Bhat, D.D.S., D.P.H., EODPP, NIDR

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This is an exploratory effort to examine potential markers of maldevelopment in a group of children with chronic motor disability of early onset and nonprogressive course (cerebral palsy, CP). It focuses on the frequency and nature of dental abnormalities in affected children. The objectives are to examine whether dental abnormalities, especially enamel defects, can serve as markers of maldevelopment, and whether such findings can provide information concerning timing of adverse events or exposures.

The significance of the research is that enamel hypoplasias and other dental anomalies can offer clues as to the timing of insults or exposures that occur from the fourth month of gestation to the age of about 12 months postnatally. Correlation of dental with clinical data may offer a means to explore the timing of departure from the normal course of development in a group of children with chronic motor disability of early onset and nonprogressive course (CP or mental retardation).

The work is now subsumed within project number Z01 NS 02863-01 NEB.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 NS 02819-03 NEB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

California Cerebral Palsy Registry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D. Medical Officer NEB, DIR, NINDS

COOPERATING UNITS (if any)

Judith Grether, Ph.D., Susan Cummins, M.D., Birth Defects Monitoring Group, Environmental Epidemiology and Toxicology Branch, Department of Health Services, California; Health Officers Association of California

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.1

PROFESSIONAL: 0.1

OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In cooperation with the California Birth Defects Monitoring Program of the California Department of Health and the March of Dimes, NEB has participated in the establishment and is sharing in the utilization of a population-based registry of children with cerebral palsy in four counties of the San Francisco Bay area for births between 1983-1985. In this birth cohort of approximately 156,000 children, 192 cases have been identified, excluding those with abnormalities related to events after the first month of life. Analysis using the examinations and birth certificates, and initial interview are in publication or preparation; those employing medical record review are imminent.

The work is now subsumed within project number Z01 NS 02863-01 NEB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02838-02 NEB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Retroviral Diseases of the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Gustavo C. Roman, M.D. Chief NEB, DIR, NINDS

COOPERATING UNITS (if any)

William A. Blattner, M.D., C. DCE, EEB, NCI; Clarence J. Gibbs Jr, Ph.D., DIR, CNSS, NINDS

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2
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CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The Neuroepidemiology Branch will begin a registry of HTLV-I infections of the nervous system to obtain data on the magnitude of this problem. Case-control studies will be undertaken to determine risk factors for the development of HAM/TSP. Patient registry should also allow future therapeutic trials.

There is also interest on the study of HIV dementia. These studies are in the planning stages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02861-01 NEB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Guillain-Barré Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Gustavo C. Roman, M.D.

Chief

NEB, DIR, NINDS

COOPERATING UNITS (if any)

Pan American Health Organization (PAHO); Peking Union Medical College (PUMC), Beijing China

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.4

PROFESSIONAL: 0.4

OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study will determine the incidence of Guillain-Barré syndrome in Latin America, as part of the Pan American Health Organization's program for poliomyelitis surveillance. Studies are also in the planning stages in the People's Republic of China.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02862-01 NEB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurocysticercosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Gustavo C. Roman, M.D. Chief NEB, DIR, NINDS

COOPERATING UNITS (if any)

Mexican National Institute of Neurology and Neurosurgery; Ecuadorean Academy of Neurosciences

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.4

PROFESSIONAL: 0.4

OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study will determine the natural history of neurocysticercosis (NCC) in endemic regions of Mexico and Ecuador, and is in the planning stages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02863-01 NEB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The California Cerebral Palsy Projects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D.

Medical Officer

NEB, DIR, NINDS

COOPERATING UNITS (if any)

Dr. Judith Grether; Dr. Susan Cummins; Birth Defects Monitoring Group, Department of Health Services, California; Health Officers Association of California

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.4

PROFESSIONAL: 0.4

OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has established a population-based registry of children with cerebral palsy (CP) in four San Francisco Bay Area counties, for study of demographic and medical characteristics as they relate to the occurrence of CP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02866-01 NEB

PERIOD COVERED

The Reliability of Diagnoses of First Seizures

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

October 1, 1991 through September 30, 1992

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Joseph M. Scheller, M.D.	Special Expert	NEB, DIR, NINDS
Others:	Stanley E. Emery, M.D.	I.P.A. Expert	NEB, DIR, NINDS
	Robert Abel, Ph.D.	Staff Fellow	BFSB, DIR, NINDS
	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS

COOPERATING UNITS (if any)

Steven Weinstein, M.D., Neurology Department, Children's Hospital National Medical Center

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	0.3	PROFESSIONAL:	0.3	OTHER:	0.0
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CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input checked="" type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We are examining the consistency of diagnosis of a first seizure in children seeking care at a multispecialty urban teaching hospital. We are investigating whether the episode described was a first seizure, a nonfebrile seizure, whether it was symptomatic of an underlying illness, and how that seizure should best be descriptively classified. Among other information sought will be the source of the medical history, training of person in medical facility who records the history, length of time from episode to recording of history. At least two versions of the history are being recorded, and a sample audiorecorded; versions of the diagnostic impressions are being compared for consistency and for patterns of any differences observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02867 NEB
PERIOD COVERED October 1, 1991 through September 20 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurologic morbidity and its antecedents within the NCPP dataset		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.: Jonas Ellenberg, Ph.D. Karin B. Nelson, M.D.	Chief Medical Officer	BFSB, DIR, NINDS NEB, DIR, NINDS
COOPERATING UNITS (if any) BFSB, NINDS; NEB, NINDS		
LAB/BRANCH Biometry and Field Studies Branch and Neuroepidemiology Branch,		
SECTION		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES)		
<div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The Collaborative Perinatal Project of the NINDS data set continues to be an important resource for information relating maternal and pregnancy and perinatal factors with neurologic outcome in the newborn and child. Current projects employing this material involve the investigation of <u>seizure disorders and motor disability in twins</u> , and the fetal heart rate monitoring by intermittent auscultation as related to neonatal and later neurologic outcome. A project on growth in cerebral palsy (CP), before and after birth, is planned.		

ANNUAL REPORT

October 1, 1991 through September 30, 1992

Clinical Neurosciences Program, DIR

National Institute of Neurological Disorders and Stroke

Neuroimmunology Branch

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Annual Report

October 1, 1991 to September 30, 1992
Clinical Neurosciences Program, DIR
National Institute of Neurological Disorders and Stroke
Neuroimmunology Branch

Dale E. McFarlin, Chief

Research in the Neuroimmunology Branch (NIB) is conducted in four administrative groups: the Office of the Chief (OC), the Neurological Disease Section (NDS), the Molecular Immunology Section (MIS), and the Cellular Immunology Section (CIS).

Clinical research is carried out in the OC and is closely integrated with basic research in the three Sections.

Over the past year, a new clinical research project, examination of natural history and therapy of multiple sclerosis (MS) using magnetic resonance imaging (MRI) (Z01 NS 02853-01 NI), has been initiated. This represents an expansion of ongoing research on using MRI to examine gadolinium-enhancing lesions in patients with early relapsing-remitting disease. Our initial study of gadolinium-enhancing lesions in six patients with mild relapsing-remitting disease showed that a considerable amount of subclinical disease activity occurred in patients with this form of MS. This conclusion was based on the observation that during the study only three clinical exacerbations occurred but 95 new enhancing lesions were detected. Many of the gadolinium-enhancing lesions evolved into persistent nonenhancing lesions detectable by an area of increased signal on T2 weighted images. Thus, the extent of pathology detected by MRI increased, over time.

The gadolinium-enhancing lesions in patients with mild relapsing-remitting MS, vary in frequency and size. Over the past year, a new approach that employs computer-assisted analysis to quantitate the total area of enhancement on each scan has been developed and incorporated into the longitudinal evaluation of patients. Significant correlation between the total area of new enhancing lesions and the number of new lesions has been observed. During these studies, it became apparent that there is considerable variation in the frequency of new gadolinium-enhancing lesions, and in many patients, the lesion frequency was not constant. Instead, there were bursts of increased lesion frequency that appeared to occur periodically. Most new MRI lesions were not associated with changes in a patient's clinical status. However, when clinical disability did worsen, this tended to coincide with bursts of enhancing lesions. Two important questions regarding the relationship between MRI changes and clinical disease activity have emerged: First, can MRI abnormalities that correlate with periods of clinical worsening be detected? Secondly, does disease activity seen on MRI relate to long-term disability? In order to address these important areas, our serial studies of gadolinium-enhancing lesions in relapsing-remitting MS have been extended and new patients added to the protocol.

Considerable enthusiasm for using MRI to assess treatment of MS currently exists among clinical investigators of demyelinating disorders; however, our observation that the frequency of gadolinium-enhanced lesions is not constant, indicates that this approach would be more complicated than initially

thought. In order to assess the best possible use of MRI as an outcome measure for clinical trials, the frequency of new enhancing lesions in 10 relapsing-remitting patients has been evaluated using a statistical method termed "boot strap analysis." The optimal design for clinical trials, the required sample sizes, and the number of monthly scans required to detect a fifty percent reduction in lesions in a hypothetical trial were determined. These studies, in collaboration with Drs. Joseph Frank of the In Vitro Magnetic Resonance Research Center and Paul Albert, BFS, NINDS, showed that a trial design using parallel groups requires both a large number of patients and MRI scans. In contrast, the findings suggested that evaluation of a given treatment could be accomplished with a crossover trial design, but six baseline MRI scans at monthly intervals would be necessary to establish lesion frequency before treatment. Using this information as background, an open trial of cyclosporine A (CSA) has been initiated in patients with relapsing-remitting MS. This will assess the concept of using MRI to evaluate treatment in MS and provide information about the use of CSA. The rationale for using CSA is that this compound inhibits lymphocyte activation, and in relapsing-remitting disease, an early event in breakdown in the blood-brain barrier (BBB) has been postulated to involve interaction between activated lymphocytes and the vascular endothelial cells (EC) that comprise the BBB.

The autoimmune unit of the NDS has continued investigation of the interaction between lymphocytes and EC in vitro in collaboration with Drs. Richard McCarron and Maria Spatz, SB, NINDS. Treatment of EC in vitro with gamma interferon (IFN- γ), interleukin-1 (IL-1), or tumor necrosis factor- α (TNF- α) significantly increases adhesion between T cells and EC. Parallel studies showed that treatment of EC with these cytokines increased expression of intracellular adhesion molecule-1 (ICAM-1). Pretreatment with transforming growth factor- β 1 (TGF- β 1) decreased adhesion, but had little effect on increased expression of ICAM induced by the cytokines. These results indicate that interaction between T cells and EC can be modified by a number of cytokines. Some of these cytokines probably act by increasing the expression of "adhesion molecules" such as ICAM-1; however, it seems likely that other as yet unidentified components may contribute to adhesion.

In order to examine the function of adhesion molecules in vivo, a series of experiments using monoclonal antibodies to modify murine experimental allergic encephalomyelitis (EAE) have been initiated. The results indicate that EAE can be decreased by administering large amounts of monoclonal antibodies specific for ICAM-1 or lymphocyte function-associated antigen-1 (LFA-1). However, when smaller amounts of these antibodies were given, EAE was augmented, and under some conditions, was lethal. It seems likely that the increase in disease severity is related to a "secondary" lymphocyte signal provided by the monoclonal antibody. The findings in EAE obviously have major implications for therapeutic proposals using monoclonal antibodies directed at adhesion molecules.

Because of findings derived from EAE, there has been considerable interest in studying the immune response to myelin antigens in MS. During the past year, CIS has continued investigation of T-cell reactivity to myelin compounds. Emphasis has been placed on the response to myelin basic protein (MBP) because of the well-established encephalitogenic properties of this protein. In addition, studies of T-cell reactivity to other myelin components, including the proteolipid protein, have been initiated. T-cell lines selected by repeated stimulation with whole MBP have been used to

characterize the major peptide epitopes. A major immunogenic region has been identified in residues 87-106. This region is highly conserved and known to be encephalitogenic in several species.

The HLA molecules that present epitopes within the 87-106 peptide and the T-cell receptors (TCR) used for recognition of these epitopes have been studied using specific cell lines derived from patients with MS and normal individuals. HLA DR2, DR4, and DR6, each of which is overrepresented in certain MS populations, were shown to present the 87-106 peptide. The fine specificity of T cells that react to this peptide was studied in detail using truncated synthetic polypeptides and alanine substituted peptides. Considerable heterogeneity in fine specificity of T-cells that react to this peptide was found. The results of a collaborative study between the CIS and the MIS on TCR usage by the CTL lines are consistent with this conclusion. In these experiments, the antigen recognition molecules for 15 peptide-specific T-cell lines were analyzed and 12 different V α and 9 different V β chains were used. Collectively, these observations indicate that the human T-cell response to a well-characterized component of myelin is quite heterogeneous. This implies that specific immunotherapies aimed at TCRs in MS would be more complicated than previously anticipated.

The relationship between viral infection and diseases of the nervous system, and particularly, viral-induced immunopathologic disease, continued to be of major interest in NIB. Over the past year, a considerable amount of clinical and fundamental research in the NIB has involved the investigation of neurologic diseases associated with HTLV-I and HTLV-II, as well as normal carriers of these viruses. Although HTLV-I is endemic to certain geographic regions, an increasing number of patients with HTLV-I-associated myelopathy/tropical spastic paraplegia (HAM/TSP) have been recognized in the United States. The NIB has been consulted about many of these. In addition, a patient with a clinical presentation indistinguishable from HAM/TSP has recently been evaluated by the NIB and shown to be infected with HTLV-II. This virus, first identified about 10 years ago, is known to be endemic in certain Native American groups and to infect certain populations of intravenous drug users. The identification of a neurologic disorder associated with HTLV-II has significant implications for screening blood to be used for transfusion, as well as the evaluation of other patients with chronic myelopathy.

Two years ago, immunologic studies of HAM/TSP, in collaboration with Drs. Anthony Fauci, NIAID and Scott Koenig, MedImmune, Inc., showed that patients with HAM/TSP have large numbers of circulating CD8+ CTL directed against the HTLV-I Tax protein. CTL blood levels were sufficiently high that these cells could be detected directly, without in vitro amplification. However, similar levels of HTLV-I-specific CTL were not found in normal carriers of the virus. This observation led to the hypothesis that an immunopathologic component contributes to the neurological abnormalities in HAM/TSP. During FY 1991, a retroviral unit was established in the NDS, and over the past year, has extended our previous observations. Studies of additional patients with HAM/TSP and normal carriers of HTLV-I have continued to show a major difference in the levels of CTL. In addition, techniques have been developed to measure the precursor frequency of circulating CTL (pCTL) in blood and cerebrospinal fluid (CSF). Comparison of the pCTL in the blood of patients with HAM/TSP normal carriers indicates that they are approximately 100-fold more frequent in patients with the neurologic disease.

Patients with HAM/TSP usually have a mild pleocytosis, and in three patients, sufficient quantities of lymphocytes have been obtained to assay CTL in the CSF. As in the blood of these same individuals, remarkably high levels (1/100-200 CD8+ cells) of pCTL specific for the Tax protein were found. The high levels of CTL in HAM/TSP patients and the reports of other investigators that the spinal cord is infiltrated by CD8+ cells are consistent with an immunopathologic basis for HAM/TSP. Because of this, a small group of patients have been treated with high doses of steroids. Substantial clinical improvement has been noted in three individuals.

Efforts to define the epitopes recognized by T cells of HAM/TSP patients are in progress. In general, the peptide sequence of the Tax protein that is recognized by CTL varies among HAM/TSP patients and correlates with the HLA class I allele that presents the peptide. In addition, some patients with HAM/TSP have CD8+ class I-restricted CTL that recognize peptides derived from other HTLV-I proteins such as the envelope glycoprotein.

Much of the fundamental research in the MIS has focused on how antigenic proteins are presented by MHC molecules, and specifically, how a given HLA class I molecule can bind a diverse array of peptides. Class I MHC molecules bind peptides within the central groove in the molecule. Six peptide-binding pockets extend from this central groove and are partly composed of polymorphic amino acid residues. In order to study the molecular basis for peptide binding, single amino acid substitutions within the various pockets of HLA-A2 have been generated by site-directed mutagenesis. These mutants have been analyzed for their effects on presentation of three different viral peptides to HLA-A2-restricted CTL specific for: 1) influenza M1 58-66; 2) human cytomegalovirus B glycoprotein 619-628; and 3) HTLV-I Tax 12-19. The results have shown that each of the different viral peptides interacts with similar pockets and residues within these pockets of the HLA-A2. Thus, many peptides with considerable differences in primary structure can bind to the same sites. A peptide-binding assay that detects direct binding of peptides to HLA molecules in vitro has been developed and is being used to define the anchor residues within these three virus peptides that are involved in MHC binding.

The MIS has also placed major emphasis on investigation of the immune response to virus at the molecular level. Elucidation of the basis of T-cell recognition of viral peptides has potential implications for the development of putative peptide vaccines. Since both class I- and class II-restricted T cells are required for induction of humoral and cellular immune responses, the MIS is interested in whether epitopes presented by both class I and class II HLA molecules exist on the same peptide. This group has recently identified an influenza virus peptide that can be presented by both class I and class II molecules. The molecular bases for interactions between this peptide and the two classes of HLA molecules have been studied in detail using alanine substitutions and truncations. The research demonstrated that the class I- and class II-restricted epitopes are not identical, but are overlapping and share a core of six amino acids. The structure of this peptide has been compared to 11 other viral antigens that can be presented by both class I and class II HLA molecules and all appear to have a common amino acid motif which provides the precise side chain contacts required for peptide binding. This motif could provide the basis for the rational design of peptide vaccines.

Although antigen presentation by HLA molecules is essential for

recognition of antigen by T cells, the expression of these molecules in the CNS is considerably less than in other tissues. The mechanisms responsible for this are unknown and a number of projects in the NIB are examining the regulation of MHC genes in the CNS. Studies with cultures of fetal cells indicate that class I MHC molecules are regulated differently in astrocytes, oligodendrocytes, and neurons. An implication of these findings is that each of these cell types varies in the capacity to function as targets for CD8+ CTL that recognize antigen in association with class I MHC molecules.

The transcription of class I genes in non-CNS cells is regulated, in part, by a series of enhancer regions, including the class I regulatory element (CRE) and the interferon consensus sequence (ICS) that are 5' to the promoter. Fetal astrocytes spontaneously express class I molecules in tissue culture and have been used to investigate the molecular mechanisms responsible for regulation of these molecules in collaboration with Dr. Keiko Ozato, LDMI, NICHD. In fetal astrocytes, in contrast to other cell types, the CRE appears to have minimal enhancer activity and may even contain negative regulatory elements. Instead, the induction of class I molecules in these cells is mediated through the ICS. Nuclear binding proteins that react with the 5' enhancer motifs have been identified using gel mobility shift assays. In non-CNS tissues, high levels of proteins that bind to the CRE region I and the ICS are expressed. These proteins are undetectable in extracts of normal brain, but are spontaneously expressed in cultured astrocytes at a time coinciding with the production of class I molecules.

HLA expression by neurons is undetectable in situ, and in order to obtain insight into the mechanisms responsible for this phenomenon, studies of human neuroblastoma (HNCL) cell lines have been initiated in collaboration with Dr. Lois Lampson at Harvard University. These HNCL are transformed, but continue to produce human neurofibrillary proteins. Initial studies showed that HLA class I molecules and $\beta 2$ microglobulin ($\beta 2m$) were not detected on the surface of the HNCL and mRNA was minimal. However, treatment with either IFN- γ or TNF- α increased mRNA steady state and led to the surface expression of both HLA class I and $\beta 2m$ molecules. Furthermore, the effect of these cytokines was additive. Gel shift assays using nuclear extracts from the HNCL showed that TNF- α induced the formation of a protein that binds to NF- κ B-like enhancer located 5' to the coding regions of class I and $\beta 2m$ genes. INF- γ stimulated the presence of nucleoproteins that reacted with different enhancer elements. These nuclear-binding proteins were not detected in untreated HNCL lines. Both the ICS and the NF- κ B enhancers were shown to positively regulate indicator genes transfected into the HNCL. These findings indicate that the low levels of class I HLA and $\beta 2m$ molecules in HNCL are due to the absence of nuclear proteins that enhance transcription. In addition, evidence for a negative regulatory element has been detected in these cell lines.

The Unit on MHC regulation, established in the OC last year, previously demonstrated that IL-1- β suppressed up-regulation of class II expression induced by IFN- γ in cell lines derived from a human glioblastoma. The function of various 5' noncoding regions have been examined with deletions in the DR α promoter coupled to indicator genes. This approach has provided evidence that the effect of IL-1- β is mediated through two regulatory elements, the X and Y boxes of the DR α promoter. Investigations of the expression of class II molecules in human capillary EC, in collaboration with Dr. Richard McCarron, SB, NINDS have been initiated. No constitutive

expression of class II molecules was detected; however, treatment with IFN- γ led to the expression of both HLA DP and HLA-DR.

A major question concerning the regulation of HLA molecules in CNS cells is, whether viral infection affects these processes? A new study has been initiated to examine this question. The units on retrovirus infection and HLA regulation have developed a method for infecting the HNCL described above with HTLV-I and are assessing the effects of this on regulation of HLA and B2m genes.

A major conclusion from research in the NIB, as well as other laboratories, is that increased expression of a variety of cell surface molecules contributes to immune reactivity in the nervous system. Elucidation of mechanisms that reduce the expression of such molecules and immune reactivity have important implications for autoimmune processes in the CNS. Studies of TGF- β 1 in chronic-relapsing EAE by the NDS are relevant to this area. Previously, this group showed that pretreatment of mice with TGF- β 2 prior to the transfer of encephalitogenic T cells prevents EAE and the administration of this cytokine after the initial episode markedly inhibits subsequent relapses. Immunologic analysis of the CNS showed less in the inflammation in mice treated with TGF- β 1 than in untreated animals, and the expression of a variety of molecules such as class II MHC (Ia) and ICAM was less in the TGF- β 1 treated mice. Thus, TGF- β 1 has an important effect on the target tissue.

TGF- β 1 belongs to a family of natural peptides with a wide spectrum of biologic activities. The endogenous mechanisms responsible for chronic-relapsing EAE originally described in NIB are unknown, and in order to determine if the production of TGF- β 1 or related peptides contributes to remission, two approaches have been pursued. First, immunohistologic examination of CNS tissue obtained from mice beginning to recover from EAE showed the presence of TGF- β 1. Secondly, it was postulated that the administration of anti-TGF- β 1 would augment the disease, if TGF- β contributes to recovery. Experiments conducted in collaboration with Dr. Michael Sporn, LC, NCI, demonstrated that mice treated with anti-TGF- β 1 had more severe disease than controls. Immunohistochemical studies of the anti-TGF- β 1-treated mice showed increased expression of MHC class II molecules and ICAM. These observations provide evidence that TGF- β 1 has an endogenous role in immune regulation. Because of these findings, three new areas of research have been initiated. The molecular mechanisms responsible for the effects of TGF- β are being analyzed; the effects of other peptides, namely TGF- β 2, are being studied. Finally, a trial of TGF- β in human demyelinating diseases is being considered.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02202-17 NI

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological Studies in Patients with Multiple Sclerosis and Other CNS Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dale E. McFarlin, M.D.	Chief	NI	DIR	NINDS
Others:				
Henry F. McFarland, M.D.	Deputy Chief	NI	DIR	NINDS
Roland Martin, M.D.	Visiting Fellow	NI	DIR	NINDS
Mary E. Smith, M.D.	Clinical Associate	NI	DIR	NINDS
Tanya Lehky, M.D.	Clinical Associate	NI	DIR	NINDS
Michael Racke, M.D.	Clinical Associate	NI	DIR	NINDS

COOPERATING UNITS (if any)

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 Nicholas Patronas, M.D., Dept. of Diagnostic Radiology, Georgetown University;
 Steven Beall, M.D., Dept. of Neurology, University of British Columbia

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

12.0

PROFESSIONAL:

8.0

OTHER:

4.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to assess multiple factors related to the pathogenesis of neurologic diseases. The research is focused on the investigation of multiple sclerosis (MS). Genetic and immunological factors which may contribute to this disorder are being evaluated in patients with sporadic disease and families with multiple affected members. Genes encoding HLA molecules and T-cell receptors in normal controls and individuals with MS are being compared. The cellular immune responses to myelin basic protein, proteolipid protein, and common human viruses are being evaluated in the same populations.

Patients with other inflammatory disorders of the central nervous system that resemble MS are being assessed. These include, systemic lupus erythematosus, other forms of vasculitis, and HTLV-I-associated myelopathy/tropical spastic paraplegia (HAM/TSP). Immunologic, clinical, and MRI findings in HAM/TSP are being compared to normal carriers of HTLV-I. Evidence of an immunopathologic component to HAM/TSP has been obtained from the detection of high levels of cytotoxic lymphocytes directed at HTLV-I in the blood. New approaches have been developed to assess cellular immune activity in the cerebrospinal fluid. Patients who have high levels of cytotoxic T cells directed at HTLV-I are being treated with a high-dose, alternate-day steroid regimen. Neurologic disorders associated with HTLV-II are being assessed, as well as normal carriers of this virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02204-17 NI
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunologic Mechanisms in Experimental Autoimmune Diseases of the Nervous System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Dale E. McFarlin, M.D. Others:	Chief Michael R. Racke, M.D. Paul Massa, Ph.D. Mary E. Smith, M.D. Paul Drew, Ph.D. Benjamin Segal, M.D.	NI DIR NINDS NI DIR NINDS Syracuse University NI DIR NINDS NI DIR NINDS NI DIR NINDS
COOPERATING UNITS (if any) Cedric S. Raine, Ph.D., Professor, Albert Einstein U.; Maria Spatz, M.D., Section Chief, SB, DIR, NINDS; Richard McCarron, Ph.D., Special Expert, SB, DIR, NINDS; Keiko Ozato, Ph.D., Section Chief, LDMI, DIR, NICHHD		
LAB/BRANCH Neuroimmunology, CNP		
SECTION Neurological Disease Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 3.5	PROFESSIONAL: 2.0	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Current research is focused on a chronic-relapsing model of <u>experimental allergic encephalomyelitis (EAE)</u>. This disease is produced by the transfer of lymphocytes sensitized against <u>MBP</u> to syngeneic mice. The neurologic dysfunction is characterized pathologically by <u>inflammation</u> and <u>primary demyelination</u>. The immunological mechanisms responsible for the initial episode and the chronic disease are being investigated. Because the migration of immune cells from the blood into the central nervous system (CNS) occurs before clinical disease, interactions between endothelial cells (EC) which form the blood-brain barrier (BBB) and immune cells are being studied <u>in vitro</u>. The function of various adhesion molecules in both adherence and lymphocyte signaling are being examined. The effects of various cytokines on lymphocyte/EC interactions and on clinical disease are being studied. <u>Astrocytes</u> and <u>microglia</u> are in close proximity to EC, and in this anatomical location, may react with immune cells. The expression of <u>major histocompatibility complex</u> (MHC) molecules in these cells and the influence of cytokines on the regulation of these molecules is being evaluated using cultures of murine fetal CNS cells, adult human glia cells, and murine brain capillary EC. Expression and regulation of MHC molecules by <u>neurons</u> is also being examined in <u>human neuroblastoma cell lines</u>. </p> <p> The administration of <u>transforming growth factor-beta1</u> (TGF-β1) prior to the onset of disease, reduces the intensity of the initial episode. When given after the first episode, the subsequent course is significantly less severe. TGF-β1 has been demonstrated by immunochemical techniques in EAE lesions which suggests that this cytokine has a regulatory function <u>in vivo</u>. The administration of <u>interferon-gamma</u> (IFN-γ), also inhibits the initial episode and it is possible that this cytokine increases the endogenous expression of TGF-β1. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02205-17 NI

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interactions Between the Human Immune System and Antigens in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Henry F. McFarland, M.D.	Deputy Chief	NI	DIR	NINDS
Others:				
Dale E. McFarlin, M.D.	Chief	NI	DIR	NINDS
William E. Biddison, Ph.D.	Section Chief	NI	DIR	NINDS
Suhayl Dhib-Jalbut, M.D.	Special Volunteer	NI	DIR	NINDS
Rhonda Voskuhl, M.D.	Clinical Associate	NI	DIR	NINDS

COOPERATING UNITS (if any)

John R. Richert, M.D., Assoc. Prof., Georgetwon U.; Diane Griffin, M.D., Ph.D., Prof., Dept., Neurology, Johns Hopkins U.; Roland Martin, M.D., Assist. Prof., Tubingen U., Germany

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:
4.5

PROFESSIONAL:
4.0

OTHER:
0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to examine the manner in which immunologic mechanisms may contribute to diseases of the nervous system. The cellular and humoral immune response to putative antigens and possible immunopathologic diseases such as multiple sclerosis (MS) are being studied. Included in these studies have been examinations of the immune response to viruses which can commonly infect the nervous system and which could be related to the induction of immunopathologic disease processes. In addition, the immune response to antigens of myelin such as myelin basic protein and proteolipid protein which may represent targets of immune-mediated diseases of myelin, has been studied. The emphasis in these studies has been on identifying differences in these immunologic responses which may occur in patients with diseases of the nervous system such as MS, as compared to healthy individuals. Particular attention has been given to examining the influence of genetic makeup on both the induction and effector phases of these immune responses. In particular, the role of HLA molecule genes and T-cell receptor genes have been investigated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02603-09 NI

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of Lymphoid Cell-Cell Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: William E. Biddison, Ph.D. Section Chief NI DIR NINDS

Others:

Ursula Utz, Ph.D. Visiting Fellow NI DIR NINDS

Tomiko Tsuchida, M.D., Ph.D. Visiting Fellow NI DIR NINDS

COOPERATING UNITS (if any)

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LAB/BRANCH

Neuroimmunology, CNP

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

6.0

PROFESSIONAL:

3.0

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The general objective of this project is to define the mechanisms by which human lymphoid cells interact with antigen-presenting cells in order to produce and regulate immune responses. Over the past year, there have been three major efforts underway that are targeted on this objective: 1) dissection of the molecular basis of viral peptide binding and presentation for T-cell recognition by HLA class I molecules; 2) analysis of antigen-presentation pathways for class I- versus class II-restricted antiviral cytotoxic T lymphocyte (CTL) responses; and 3) delineation of the heterogeneity of expressed T-cell receptor (TCR) genes in myelin basic protein (MBP)-reactive T cells obtained from multiple sclerosis (MS) patients. The principle findings are as follow: 1) class I- and class II-restricted T cells can recognize structurally similar, but distinct epitopes of the same viral peptide, and such peptides share a common motif with other peptides that are also recognized by class I- and class II-restricted T cells; 2) common structural features of HLA-A2 molecules can determine the binding and presentation of three diverse viral peptides derived from HTLV-I, human cytomegalovirus (HCMV), and influenza virus; 3) fragments of HLA heavy chains can be successfully expressed in E. coli and utilized together with iodinated β -2 microglobulin (β 2m) and peptides to produce a highly specific peptide-binding assay for class I molecules; 4) class II presentation of a cytosolic viral protein can occur in mutant cells that lack HLA-encoded ATP-dependent transporter molecules, whereas class II presentation of a short cytosolic peptide derived from that same viral protein is dependent on such a transporter; and 5) MS patients develop CD4+ CTL responses in vitro to the immunodominant MBP peptide 87-106 that are very heterogeneous at the level of fine specificity, HLA restriction, and TCR V α and V β usage.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02817-03 NI

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Involvement of Human Retrovirus Associated with Chronic Neurologic Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Steven Jacobson, Ph.D. Section Chief NI DIR NINDS

Others:

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Irina Elovaara, M.D.	Special Volunteer	NI	DIR	NINDS
Tanya Lehky, M.D.	Clinical Associate	NI	DIR	NINDS
Scott Koenig, M.D.	Head, Immunology	MedImmune, Inc.		
Cedric S. Raine, Ph.D.	Professor	Albert Einstein U.		

COOPERATING UNITS (if any)

William Blattner, M.D., Chief, VES, NCI; Bernard Poiesz, M.D., Chief, Dept. of Medicine and Microbiology, SUNY Health Sciences Center; Thomas Waldmann, Chief, MET Branch, NCI; Anthony Fauci, M.D., Director, NIAID

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Neurological Disease Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

2.5

OTHER:

2.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project are to define the roles of human retroviruses and host cellular immune responses directed at these agents in chronic-progressive neurologic disease. The association of the human T lymphotropic virus type I (HTLV-I) and the disorder known as HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is being used as the model system for an understanding of the pathogenic mechanism(s) involved in human retroviral diseases of the central nervous system (CNS). These investigations involve the virologic and molecular characterization of the agent(s) isolated from patients with neurologic disease, because unique features of these viruses may contribute to the pathogenesis of CNS disease. Long-term T and B cell lines expressing endogenous human retrovirus have been established from affected individuals and have been used to characterize these molecular and virologic aspects of these agents. The presence of human retrovirus in the CNS of these patients is being assessed by in situ hybridization.

Host cellular immune responses to various strains of HTLV-I are characterized because immunopathologic mechanisms are believed to be directly involved in the pathogenesis of HAM/TSP. Focus is being placed on the role of cytotoxic T cells (CTL) in the pathogenesis of HAM/TSP because circulating CTL have been demonstrated from the peripheral blood and cerebrospinal fluid of HAM/TSP patients, but not from HTLV-I carriers. The precursor frequency of these CTL is being assessed by limiting dilution studies. Lastly, an association has been documented between HTLV-II, a closely related retrovirus to HTLV-I, and a chronic-progressive neurologic disease clinically indistinguishable from HAM/TSP.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02831-02 NI

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Class II Major Histocompatibility Complex Genes in the CNS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Elliot P. Cowan, Ph.D. Special Expert NI DIR NINDS

Others:

Suhayl Dhib-Jalbut, M.D. Special Volunteer NI DIR NINDS

Richard McCarron, Ph.D. Special Expert SB DIR NINDS

COOPERATING UNITS (if any)

Jeremy M. Boss, Ph.D., Dept. of Micro. and Immunol., Emory University School of Medicine; Lois Lampson, Ph.D., Harvard University

LAB/BRANCH

Neuroimmunology, CNP

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Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

3.5

2.5

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

HLA class II molecules are required for the presentation of antigen to CD4+ T cells in the induction of immune reactivity and for target recognition by effector T-lymphocytes. Aberrant expression of class II genes has been postulated as a factor in the generation of organ-specific autoimmune disease. While not normally expressed on certain cell types in the central nervous system (CNS), class II can be induced on many of these same cells by various cytokines or infection with viruses. The purpose of this project is to understand molecular genetic mechanisms that control the expression of HLA class II genes in the CNS. Studies over the past year have examined three of these systems: 1) identification of class II gene-enhancer elements that are required for suppression of class II expression in a glioblastoma cell line by interleukin-1 β (IL-1 β); 2) the up-regulation of class II expression on normal human cerebral microvessel endothelial cells by interferon- γ ; and 3) the induction of class II expression on human neuroblastomas by infection with HTLV-I. The major findings are as follows: 1) Evidence has been obtained that indicates the DR α gene requires the X box, in addition to the Y box, for a negative response to IL-1 β . 2) Human cerebral microvessel endothelial cells express HLA-class II in response to IFN- γ treatment. 3) The infection of human neuroblastomas with HTLV-I cocultured with a chronically infected T-cell line, results in the induction of class II transcripts and cell surface class II expression (HLA-DR, -DQ, and -DP).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 NS 02853-01 NI

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Examination of Natural History and Therapy of Multiple Sclerosis Using MRI

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Henry F. McFarland, M.D.	Deputy Chief	NI	DIR	NINDS
Others:				
Dale E. McFarlin, M.D.	Chief	NI	DIR	NINDS
Lael Stone, M.D.	Special Volunteer	NI	DIR	NINDS
Suhayl Dhib-Jalbut, M.D.	Special Volunteer	NI	DIR	NINDS
Michael K. Racke, M.D.	Clinical Associate	NI	DIR	NINDS
Tanya J. Lecky, M.D.	Clinical Associate	NI	DIR	NINDS
Joseph A. Frank, M.D.	Acting Director	DRPP		OD

COOPERATING UNITS (if any)

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

3.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to use magnetic resonance imaging (MRI) to examine the natural history and potential therapeutic approaches in multiple sclerosis (MS). Emphasis has been placed on investigation of the early MS lesion which is characterized by enhancement on T1 weighted MRI images following administration of gadolinium-DTPA. Results from initial studies have indicated that MS can be an active disease, even during periods of remission in the early relapsing-remitting phase of the illness. Frequency of gadolinium-enhancing lesions has been examined in a series of patients with mild early MS. Monthly MRIs done longitudinally have been used to determine the optimal approach for using MRI as an outcome measure in MS. Possible correlations between clinical changes and both the frequency and area of gadolinium-enhancing lesions has been examined in order to determine if relationships between disease activity and changes on MRI exist.

ANNUAL REPORT

October 1, 1991 through September 30, 1992

Surgical Neurology Branch - Clinical Neurosciences Program, DIR

National Institute of Neurological Disorders and Stroke
Edward H. Oldfield, M.D., Chief

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ANNUAL REPORT
October 1, 1991 through September 30, 1992
Surgical Neurology Branch, Division of Intramural Research
Clinical Neurosciences Program
National Institute of Neurological Disorders and Stroke

Edward H. Oldfield, M.D., Chief

The Surgical Neurology Branch (SNB) is composed of the following: I) Clinical Neurosurgery Section, A. Tumor Biology Unit, B. Central Nervous System Implantation Unit, C. Molecular Biology Unit; and the II) Biochemistry Section.

The SNB has as its major research functions: (1) to study the biology and therapeutic approaches to malignant and benign tumors of the brain and pituitary gland; (2) investigate the capability of implanted tissues into the brain of animals and humans to survive and integrate anatomically or biochemically with the host brain to influence brain function; (3) to investigate certain vascular disorders of the central nervous system (CNS); and (4) investigation and surgically treat patients with epilepsy. The Branch's clinical function is to provide the neurosurgical services to its own research protocol patients and to patients seen in consultation in the NIH, Clinical Center. The SNB is located in Buildings 9 and 10. Its staff includes clinical neurosurgeons at various levels of training and experience, as well as senior and junior scientists, and a support staff of scientific, technical and administrative individuals.

In addition to its primary functions of clinical and basic research, the SNB provides young neurosurgeons with experience in clinical and laboratory investigation in a combined clinical and neuroscience environment. Of those who have participated in the SNB program as Medical Staff Fellows and Senior Staff Fellows, many have entered academic positions in neurosurgery.

I. CLINICAL NEUROSURGERY SECTION

Edward H. Oldfield, M.D., Chief

The clinical activities of the Surgical Neurology Branch include investigating the biologic behavior and mechanisms of the pathophysiology of malignant primary brain tumors, pituitary tumors, certain vascular disorders of the CNS, surgical management of epilepsy refractive to medical therapy, and tissue implantation in the CNS to reverse neurologic disorders.

Primary Brain Tumors

Clinical Use of Immunotoxins to Treat CNS Tumors

A Phase I clinical trial was completed to investigate the clinical use of immunotoxins in the treatment of leptomeningeal carcinomatosis. The immunotoxin, a conjugate of a human antitransferrin receptor antibody with ricin A chain, was administered in single injections (1.2 to 1200 μ g) in patients where leptomeningeal carcinomatosis was refractory to current therapies. Eight patients were treated. No toxicity occurred until the highest doses were reached. Bioassays of cerebrospinal fluid (CSF) samples and a reduction in tumor cells in the CSF in 4 patients indicated *in vivo* antitumor activity at tolerable doses. The pharmacokinetics of immunotoxins in the CSF after intraventricular injection was established.

Methods were investigated for direct injection into the brain and into CNS tumors, to achieve an homogeneous distribution in the brain of drugs which do not cross the blood-brain barrier (BBB). A technique was perfected to successfully directly infuse materials into the interstitial space of the brain and to use convection to greatly enhance the distribution of large and small molecules within the brain parenchyma.

Disruption of the Blood-Brain Barrier

We completed two projects which quantified permeability of the BBB in the primate brain before and after barrier disruption by intracarotid mannitol using PET scanning. In a study of baboons, the permeability across normal brain vessels in the gray and white matter was measured repeatedly and the duration and extent of enhancement of vascular permeability was determined. A similar study in humans permitted quantification of vascular permeability in primary brain tumors, brain regions around the tumor, and normal brain. The results of these studies permit rational design of approaches to deliver drugs of various types to the brain using this technique.

Barbiturates as Radioprotectants of the CNS

Investigation of the radioprotective effect of pentobarbital in a primate model of cerebral radiation toxicity was carried out. A concern remains as to the applicability of a model of acute radiation toxicity in a rodent to the radiation toxicity seen in humans with cerebral radiation injury due to treatment of brain tumors. A primate model was designed to better approximate the injury seen in

humans, which is more delayed in onset. A long-term primate study is ongoing at this time

In an additional primate study, the potential radiation protective effect of pentobarbital is being examined in 12 monkeys which received multiple fractions of radiotherapy, fractionation analogous to that used clinically. Information on the basic mechanism(s) of radiation damage of the brain and hypothalamic-pituitary axis will also be obtained.

Studies of Patients with von Hippel-Lindau Disease

Von Hippel-Lindau (VHL) disease is an inherited disorder in which patients suffer from hemangioblastomas of the retina, cerebellum and spinal cord as well as renal cell carcinomas, pheochromocytomas, and visceral cysts. The disease is passed in an autosomal dominant pattern, which has been linked by recombinant fragment length polymorphism analysis to the short arm of the third chromosome. The defective gene is in the 3p 14 region.

With Drs. Berton Zbar, Gladys Glenn and Marston Linehan of the NCI, using DNA polymorphism analysis for screening for VHL disease was successful with 96% accuracy. Distinctive feeder types were identified among families with VHL disease suggesting that more than one mutant allele exists at the VHL gene locus.

In collaboration with Dr. Zbar of the NCI, we studied 13 tumors from 5 patients, analyzing them for loss of alleles on the third chromosome. These tumors demonstrate loss of one copy of the VHL allele. However, in the tumor cells, the loss is of the normal, wild type allele inherited from the normal parent. This loss of the balancing wild type allele leaves the tumor with only the abnormal allele inherited from the parent carrying VHL disease. This supports the theory that the tumors in VHL disease are caused by the loss of a tumor suppressor gene similar to that seen with retinoblastoma and type II neurofibromatosis; the affected parent contributes a defective copy of the gene allowing cells at risk to develop tumors when the other copy, the balancing wild type gene from the normal parent, is lost or rendered nonfunctional.

The utility of ultrasound with Doppler color-flow imaging to localize small tumors in the spinal cord, cerebellum, and brainstem during surgery was established. Disappearance of the syringomyelia cavity associated with hemangioblastoma was demonstrated. Thus, it was established that the syringomyelia often associated with spinal hemangioblastomas needs no separate treatment, as it will remit with successful treatment of the tumor alone.

Establishing the Physiology of Syringomyelia

Syringomyelia results in advancing paralysis, sensory loss, and pain. This study seeks to elucidate the mechanism of progression of "communicating" syringomyelia. "Communicating syringomyelia" accompanies abnormalities at the craniocervical junction. Patients are investigated preoperatively with cine- MRI scans to evaluate the movement of the fluid within the CSF spaces and the syrinx during the cardiac cycle. Pressures within the syrinx and the lumbar subarachnoid space are measured simultaneously during the resting state and during various physiologic maneuvers, such as occlusion of the jugular veins and the Valsalva maneuver. During surgery, pressures are obtained from the ventricular system, lumbar subarachnoid space, and

syntx cavity. These pressures are measured simultaneously while the patient undergoes decompression of the cerebellar tonsils at the level of the foramen magnum. Movement of the walls of the syntx cavity and the cerebellar tonsils are monitored by intraoperative ultrasound before and after the dura is opened. The images are linked in time with the pressure measurements from the various CSF spaces. Preliminary observations indicate the presence of a new, previously unrecognized, mechanism of progression of syringomyelia in these patients. The findings also indicate that it should be possible to establish when during surgery the point has been reached to sufficiently resolve the syntx postoperatively. There has previously been no way to establish the extent of surgery required to successfully treat these patients.

Gene Transfer to Treat Malignant Brain Tumors

This novel approach makes use of *in situ* gene transfer into brain tumors using a murine retroviral vector (MoMLV) which is continuously produced by genetically-engineered murine fibroblasts (vector-producer cells).

In collaboration with Drs. Blaese and Culver from the Metabolism Branch of NCI, we have developed the application of this approach in the rat brain tumor model to transfer the herpes simplex thymidine kinase gene (HStk) as a "suicide" gene which confers sensitivity of the transduced tumor cells to the antiviral drug ganciclovir (GCV). We have shown that it is feasible to induce complete tumor regression in treated animals immediately following GCV therapy. Long-term toxicity studies in mice, rats, and nonhuman primates established the safety of this approach.

The exact mechanism of tumor regression is unclear. While only a fraction of the tumor cells acquire the foreign gene, complete tumor eradication can be achieved. The production of toxic by-products of thymidine kinase/GCV interaction are perhaps responsible for such a "bystander" effect by interfering with DNA synthesis of neighboring nontransduced tumor cells. Transduction of endothelial cells and reduction of blood supply to the tumor may be another mechanism. Experiments done in collaboration with Dr. Thomas Shawker of the Radiology Department, CC, using ultrasound and Doppler color-flow imaging of treated tumors have shown a significant reduction of tumor vasculature during the early phases of GCV therapy after *in situ* transduction of the tumor with HStk.

Based on these experiments, a clinical trial to treatment of patients with malignant brain tumors using *in situ* transduction with the HStk gene and GCV therapy will be initiated in November, 1992.

Regional Therapy with Tf-CRM107 to Treat Recurrent Brain Tumors

We are investigating a new experimental approach to treat malignant brain tumors which utilizes a new class of anti-cancer compounds, targeted protein toxins. A targeted protein toxin is a compound that consists of two parts. The binding moiety targets the conjugate to the surface of tumor cells bearing the appropriate antigen or receptor, and the toxin moiety then penetrates the cell membrane and inactivates protein synthesis, resulting in cell death.

We have studied the potential of using two such targeted protein toxins for treatment of brain tumors: 1) transferrin-CRM107 (Tf-CRM107), a conjugate of human transferrin (Tf) and diphtheria toxin with a point mutation (CRM107); and 2) 454A12MAB-rRA, a conjugate of a monoclonal antibody (454A12MAB) to the human transferrin receptor and recombinant ricin A chain (rRA), the enzymatically active subunit of the ricin protein toxin. The transferrin receptor is overexpressed by all rapidly dividing cells, including tumor cells; glioblastoma cells express a greater density of this receptor than surrounding neurons and glia. We have shown that both Tf-CRM107 and 454A12MAB-rRA exhibit potent, specific killing of human glioblastoma, medulloblastoma and breast carcinoma lines in vitro. Protein synthesis was inhibited and tumor cells killed at toxin-conjugate concentrations as low as 10^{-12} to 10^{-10} M. This is well below concentrations of conjugates required in brain or in the CSF that produce local CNS or systemic toxicity. Such toxicity has been characterized both clinically and pathologically in our prior human clinical trials as well as in animal studies in rats, guinea pigs, and monkeys.

Additional evidence supporting the potential efficacy of these reagents for malignant brain tumors was obtained in a nude mouse model of human glioblastoma. We demonstrated that Tf-CRM107 and 454A12MAB-rRA inhibit tumor growth and cause tumor regression when delivered intratumorally into solid U251 human glioblastoma tumors grown in the flank of nude mice. Tf-CRM107, the more potent reagent, caused tumor regression in a dose-dependent manner.

We have now initiated a phase I trial of regional therapy with Tf-CRM107 to treat recurrent malignant brain tumors. In this clinical trial, Tf-CRM107 is delivered by slow intra- and peritumoral infusion. The purpose of this study is to determine if this new experimental compound, Tf-CRM107, can be used safely in patients with malignant brain tumors. We also hope to learn about its ability to kill tumor cells in patients with recurrent malignant brain tumors.

Pituitary Tumors

Venous Sampling to Establish the Diagnosis and Location of Hormone-Secreting Pituitary Microadenomas

We have investigated bilateral and simultaneous inferior petrosal sinus sampling in over 550 patients with Cushing's syndrome. The results are used to (1) confirm the diagnosis of Cushing's disease preoperatively, and (2) determine in which half of the pituitary gland a microadenoma resides. The study has been particularly rewarding and has demonstrated the following: (1) simultaneous sampling from both inferior petrosal sinuses with corticotrophin-releasing hormone (CRH) injection consistently distinguishes patients with Cushing's disease from those with ectopic ACTH syndrome (100% accuracy); (2) sampling from a single inferior petrosal sinus, as has previously been the general practice to establish the diagnosis of Cushing's syndrome, may be misleading and could result in an incorrect assumption of the source of excess ACTH secretion in as many as 41% of patients with Cushing's syndrome; and (3) preoperative sampling for ACTH concentrations in the inferior petrosal sinuses determines the site of ACTH-secreting microadenomas within the pituitary gland with about 70% accuracy. Therefore, bilateral sampling permits the surgeon's search for small microadenomas to be focused on one side of the gland, which should be helpful in finding smaller tumors. If no tumor is found, the half of the gland containing the microadenoma can be removed. This technique

is now being widely employed in the evaluation of patients with Cushing's syndrome.

Radiologic Imaging and Cushing's Syndrome

To evaluate the MRI scan as a means of aiding the differential diagnosis of patients with Cushing's syndrome and as a mechanism for tumor localization pre-operatively, we prospectively evaluated the results of MRI scanning in 67 patients with Cushing's syndrome. The diagnostic accuracy of MRI scanning with gadolinium - DTPA contrast was only 55%. The conclusion of this study was that MRI scanning is only occasionally helpful in locating, preoperatively, the site of an ACTH-secreting pituitary microadenoma, and is therefore only rarely beneficial as a diagnostic aid in the differential diagnosis of patients with Cushing's syndrome.

Autopsy studies have indicated an incidence of pituitary adenomas in asymptomatic patients to be 7-33%. To establish the incidence of MRI abnormalities in the general population consistent with a diagnosis of a pituitary adenoma, we studied 100 normal subjects with and without gadolinium-enhanced MRI scans. The results of this study indicate that 11% of the normal population have pituitary lesions that appear to be pituitary adenomas by MRI scanning. These results indicate that an appreciable percentage of normal adults have pituitary adenomas, and demonstrate that positive findings on an MRI in a patient with endocrinopathy do not necessarily establish that the source of the endocrinopathy is in the pituitary gland.

Cerebral Vascular Disease

Role of Endothelial-Derived Relaxation Factor (EDRF Nitric Oxide) in Regulating Cerebral Blood Flow (CBF)

The role of nitric oxide in the chemo- and autoregulation of CBF was investigated in primates. Vascular anatomy was monitored with high-resolution magnified cerebral arteriography. CBF was monitored continuously using a thermal dilution technique in which the probe was placed over the parietal cortex in the distribution of the middle cerebral artery. Vasodilatation in response to hypercarbia was demonstrated to be dependent on nitric oxide action, as increases in CBF in response to hypercarbia were completely blocked by intracarotid infusions of compounds which block nitric oxide production. Similarly, vasodilatation in response to severe hypotension was blocked by pharmacologically blocking nitric oxide production. Furthermore, basal CBF was reduced by about 20% by the intracarotid infusion of these agents. Results of these studies clearly indicate that vasodilation associated with physiologic regulation of CBF depends on production of and is mediated by nitric oxide.

Cerebral Vasospasm

The primate model cerebral vasospasm was used to investigate the etiology of delayed cerebral vasospasm after subarachnoid hemorrhage. Despite many years of investigation, the component of blood that was responsible for vasospasm had not been established. By placing isolated components of the peripheral blood around

the middle cerebral artery in a collagen clot, we demonstrated that red blood cells were essential for production of cerebral vasospasm in primates. Neuropeptide Y, which had been implicated in the etiology of cerebral vasospasm, was shown not to be associated with the onset of delayed cerebral vasospasm. Proliferation of the constituents of the arterial wall producing obstruction of the artery has also been proposed as a mechanism of delayed cerebral vasospasm in humans after subarachnoid hemorrhage. To investigate this in primates, monkeys received continuous infusion of bromodeoxyuridine intravenously for 2 weeks after subarachnoid hemorrhage. The potential of the loss of nitric oxide activity as the mechanism of delayed cerebral vasospasm was also investigated. The total loss of nitric oxide synthase in the nerve plexus surrounding the middle cerebral artery was demonstrated in animals who had cerebral vasospasm after subarachnoid hemorrhage. In contrast, when animals with delayed cerebral vasospasm recovered, immunohistochemical staining of the middle cerebral artery demonstrated recovery of staining for nitric oxide synthase. Furthermore, intracarotid infusions of L-arginine, the substrate for nitric oxide production, increased CBF in the distribution in the involved artery during cerebral vasospasm.

Dural Arteriovenous Fistulas

A subtype of cranial dural arteriovenous (AV) malformations was identified which could be detected by preoperative cerebral arteriograms and which could be successfully treated by simple interruption of the vessel draining the dural AV fistula as it entered the subarachnoid space. This observation should permit care of these patients to be treated by a surgical procedure which is much less extensive, and which is safer, than the current techniques.

The basis of the development of spinal dural AV fistulas, now recognized to be the most common type of spinal AV malformation, was investigated. Spinal dural AV fistulas were excised en block, the vessel supplying the dural AV fistula (the dural artery) was cannulated and resolution, magnified micro-arteriograms were performed. The arteriograms demonstrated a simple AV fistula in the dura without an intervening nidus of abnormal blood vessels, indicating that these lesions are probably acquired, and not congenital, as they were previously been considered to be.

Epilepsy

The surgical arm of the NINDS epilepsy program seeks to develop surgical techniques that allow more accurate localization and safer resection of epileptogenic foci than can be achieved with methods now available. The development and implementation of surgical treatments for patients whose seizures are intractable to currently available medical and surgical therapies are the immediate and long-term goals of this program.

Special subdural surface electrodes designed and built at NIH in collaboration with the BEI Branch of DRS are implanted in selected patients to obtain EEG recordings directly from the cortical surface for much longer periods than is possible during intraoperative electrocorticography. During the period of implantation, these electrodes are also utilized for focal cortical stimulation to discriminate areas that can be safely resected from areas critical for motor, sensory, language, memory-

related and other functions. Such discrimination is crucial in the topographic identification of overlap between critical cortical areas and epileptic foci during surgical procedures.

A subset of patients with epileptogenic foci originating in the language dominant hemisphere are undergoing implantation of a new type of subdural electrode designed and built at NIH. Recordings and functional stimulation mapping utilizing these electrodes during a period preceding resective surgery should allow maximum, and safer, surgical resection to be performed under general anesthesia in these patients, who are difficult or impossible to cure using current approaches. During the past year, a novel language area has been identified and located within the cortex of the basal temporal lobe. We are working to anatomically and functionally define this basal temporal language area.

Analysis of data from several recently developed methods for localizing epileptic foci is being performed to compare the sensitivity and reliability of the various techniques. During surgery for focal epilepsy, depth and special subdural (surface cortical) electrodes are being used to record from deep structures that are inaccessible by routine electrocorticography, to identify and confirm areas of potential epileptogenic activity suggested by preoperative investigation with PET scanning, magnetoencephalography (MEG), MRI, and magnetic resonance spectroscopy (MRS).

MEG offers the possibility of localizing of abnormal seizure-related electromagnetic phenomena in 3 dimensions. No other methodology currently available can noninvasively acquire this type of information, which is highly desirable for identifying epileptogenic foci for surgical excision and to study basic mechanisms in epilepsy. In several patients, we predicted correctly the localization of foci causing complex partial seizures by using MEG data in patients whose preoperative EEG (in retrospect) gave misleading and/or false localizing information. These foci, which were successfully extirpated, would have been missed if a standard temporal lobectomy had been performed.

In selected patients, subdural electrodes are modified so that in addition to their recording capability, they create a current dipole. This dipole can then be detected by MEG, allowing direct assessment of the ability of the MEG to accurately and precisely predict the location of a current dipole source within the human cranium.

An approach that should yield information about chemical changes which occur in tissues in which seizures arise is magnetic resonance spectroscopy (in vivo NMR spectroscopy). Under a new protocol, pH and lactate measurements are taken from specific regions of interest in seizure patients and are compared with measurements from similar regions in the opposite hemisphere and with measurements in normal volunteers. This study is being done in collaboration with Drs. Di Chiro and Alger, Neuroimaging Branch.

Development and Testing of a Visual Prosthetic Device for the Blind

The purpose of this study is the development, implantation and feasibility testing of a visual prosthetic device for the blind based on intracortical microstimulation of the visual cortex.

Localized electrical stimulation of the visual cortex evokes topographically-mapped visual sensations called phosphenes. Previous experiments utilizing stimulation through the pial cortical surface of the visual cortex indicate that a useful prosthesis based on surface stimulation is not feasible, primarily because of phosphene interactions. It is likely that the relatively large current levels (milliamp range) needed to elicit phosphenes with surface stimulation excite large pools of neurons, limiting the number of potential phosphenes and leading to phosphene fusion.

For several years, intramural investigators in the Surgical Neurology Branch and the Laboratory of Neural Control and the Neural Prosthesis Program of the NINDS have been working on the development of a prosthetic device based on intracortical microstimulation of the visual cortex.

Special microelectrodes of activated iridium microwelded to gold leads were designed and fabricated by investigators in the Laboratory of Neural Control. After acute and chronic studies in animals, including nonhuman primates, the custom-designed microelectrodes were tested in three acute (less than one hour per experiment) experiments. Stimulation of the visual cortex through these microelectrodes generated phosphenes in the sighted patients at 20-200 microamps.

In view of the findings from these experiments, we planned to implant an array of microelectrodes in the visual cortex of a blind patient-volunteer for three to four months. Stimulation experiments were used to evaluate the generation of phosphenes with parameters such as pulse width, pulse duration, current amplitude and pulse train length, while remaining within the predicted limits of electrochemical safety. 38 microelectrodes were implanted in the visual cortex of a patient-volunteer. Over the next four months the electrodes were stimulated 5 days per week during testing sessions. Phosphenes were elicited by stimulation of 34 of the 38 electrodes. Thresholds ranged from 2 to 20 microamps with biphasic, cathodic first, capacitor-coupled pulses. Thresholds remained stable over the duration of the study.

Phosphenes often color near threshold and were white at higher levels of stimulation. All phosphenes were in the contralateral hemifield. Phosphenes appeared within 200 msec of the onset of stimulation and, with rare exception, extinguished immediately upon cessation of stimulation. Up to 6 simultaneous phosphenes could be identified. At the termination of the experiment, the electrode arrays and were removed. Minimal scarring around the gold leads overlying the pia was identified. The next experiment planned in this project will be implantation of up to 200 microelectrodes in the visual cortex of a patient-volunteer. A system is being designed that should allow all microelectrode leads to be attached to a series of connectors mounted to the skull. If the results of these experiments are encouraging, we will attempt to provide a function to the blind patient-volunteer by interfacing the implant with a portable television camera and image processing electronics.

A. TUMOR BIOLOGY UNIT

The focus of research in this unit is a secreted protein factor known both as vascular permeability factor and as vascular endothelial growth factor (VPF/VEGF). As the name implies, this protein is unique in that it has the capability of inducing

both endothelial mitogenesis and angiogenesis, and capillary permeability. VPF/VEGF, a secreted 34-43 kDa dimeric glycoprotein, has been identified from the conditioned medium of several cell lines, and may play a role in the development and maintenance of normal and tumor-associated vasculature. Cloning studies from several sources demonstrate that this factor exhibits extensive interspecies homology, as well as some homology to PDGF. Alternative splicing of the human VPF/VEGF gene transcript produces at least three mRNA forms that code for mature polypeptides of 189, 165, and 121 amino acids. The physiologic significance of these multiple forms and the patterns of their expression *in vivo* are not known. VPF/VEGF is distinct among other identified growth factors (EGF, aFGF, bFGF, IL-1, TGF β , and PDGF) in that its mitogenic activity is specific for endothelial cells and that it is the only growth factor which exhibits vascular permeability-inducing activity.

Although this factor has been suggested to be a mediator of tumor-associated angiogenesis and capillary hyperpermeability, extensive studies of VPF/VEGF gene expression in tumor tissues have yet to be performed. The unique combination of angiogenic and vascular permeability activities within the same protein is of particular interest to the study of human CNS neoplasms. We have identified VPF/VEGF in human brain tumor cyst fluids and have determined that human and rat brain tumor cells secrete VPF/VEGF. This is a significant finding because much of the morbidity and mortality of malignant, and certain benign, CNS neoplasms is related to the degree of tumor vascularity and the extent of peritumoral vasogenic cerebral edema. Tumor vessels, which arise from angiogenesis, comprise a substantial component of some CNS neoplasms. These vessels are often fragile, and exhibit a tendency toward intratumoral hemorrhages. In addition, tumor vessels often lack the tight junctions which are normally present in cerebral microvessels and which constitute a major barrier to the development of vasogenic edema. The release of vasoactive substances by the tumor cells may also contribute to the development of vasogenic cerebral edema by direct actions on the tumor-associated endothelium. This edema is presumably a consequence of increased permeability of tumor-associated capillaries. However, the mechanism of this phenomenon is not well understood.

Although VPF/VEGF may play a role in brain tumor-associated angiogenesis and in the abnormal increase in capillary permeability observed in the tumor vessels, the factor is also found in cultures derived from certain normal cell types, including the pituitary. These findings suggest that VPF may be important in the physiology of normal vasculature. Therefore, our current studies are directed toward answering the following questions. What is the role of VPF/VEGF in the development of brain tumor-associated neovasculature and edema? Does VPF/VEGF have a function in normal brain? What is the mechanism of action of VPF/VEGF on tumor-associated and normal CNS vasculature? To address these issues we use several methodologies: molecular biology, protein biochemistry, immunochemistry, cell culture and animal models.

In a study of the levels of VPF/VEGF mRNA in 42 CNS neoplasms and 7 normal human brain samples, significantly higher levels (up to 10-fold higher) were observed in those tumors commonly associated with extensive vascularity or cerebral edema (glioblastoma multiforme, hemangioblastomas, meningiomas). In those tumors that are not associated with increased vascularity and edema (pituitary adenomas and nonastrocytic gliomas), the levels of VPF/VEGF were not significantly different from those in normal brain. Cloning and sequencing of PCR-amplified GBM and normal brain cDNA demonstrated three forms of the VPF/VEGF coding region corresponding to mature polypeptides of 189, 165, and 121 amino acids. The

relative abundance of the forms of VPF/VEGF mRNA was consistent in tumor and normal brain. Adsorption of capillary permeability activity from human glioblastoma multiforme (GBM) cell conditioned medium and GBM cyst fluids by anti VPF/VEGF antibodies demonstrated that VPF/VEGF is secreted by GBM cells and is present in sufficient quantities in vivo to induce vascular permeability. The discovery that brain tumors secrete a permeability-enhancing substance may help to explain the increased permeability in tumor-associated vessels. Our finding that the permeability-enhancing activity of VPF is inhibited by steroids correlates with the clinical observation that high-dose steroids are a successful treatment for brain tumor-associated edema.

To identify the presence and distribution of VPF/VEGF mRNA expression in normal tissues, we performed Northern blot and in situ hybridization analyses on adult rat brain, kidney, liver, lung, and spleen. By determining the sites of VPF/VEGF expression in vivo we hoped to achieve additional insight into the role of this factor in normal physiology and into the mechanisms of angiogenesis and vessel permeability. On Northern blots, the relative abundance of VPF/VEGF mRNA observed in these tissues was highest in the lung and lowest in the spleen. As determined by in situ hybridization, the patterns of VPF/VEGF expression are organ-specific. Our results demonstrated that VPF/VEGF mRNA is expressed in vivo in several tissues of the normal healthy adult rat with organ-distinct histologic distribution.

The widespread expression and organ-specific distribution of VPF/VEGF mRNA in normal rat tissues, and the increased expression in human CNS tumors, suggest an extensive role for this factor in the physiology of both normal and tumor vasculature. Through these studies we hope to gain further insight into the mechanism of action of VPF/VEGF, and the possibility of regulating its production and physiologic effects, for the purpose of improving treatment.

B. CNS Implantation Unit

Tissue Implantation in Parkinsonian Animal Models

The effects of tissue implants into the caudate nucleus of Parkinsonian animals is being studied. The models used are the hemiparkinsonian monkey and a new rat model of Parkinson's disease developed in our laboratory. Our previous work showed that grafts of fetal mesencephalon led to rapid, nearly complete recovery from the motor deficits in monkeys, while nondopaminergic fetal grafts also produced moderate motor recovery. In contrast, adrenal allografts, adrenal autografts, nondopaminergic adult tissues, and surgical cavitation alone all lead to modest behavioral recovery, which requires months to occur. One phenomenon which we have observed to varying degrees after all implantation and after cavitation alone is sprouting of intact host dopaminergic fibers, presumably from the mesolimbic dopamine system. Efforts to trace the sprouted fibers using retrograde and anterograde tracers are underway.

To further investigate this sprouting, we implanted fetal monkey amnion (which produces a neurite-promoting factor in vitro) into hemiparkinsonian monkeys. We observed significant behavioral improvement. Term amnion, which is much more readily accessible than fetal amnion, was also implanted, but the behavioral response was minimal. We have also developed an in vivo microdialysis

system to study biochemical changes in the brain of implanted monkeys. The system is useful to compare the normal and hemiparkinsonian sides of the brain and to detect increased dopaminergic activity after tissue implantation. Using this system we are able to assess in vivo changes in various brain neurotransmitters.

We have performed cell counts of dopaminergic neurons in the midbrain of normal and hemiparkinsonian monkeys. The pattern of cell loss after MPTP suggests that some of the nuclei included in the mesolimbic system are resistant to MPTP and are functionally unrelated to the substantia nigra.

Implantation in a Selective Rat Model of Parkinsonism

To provide a small animal model to perform implantation experiments, we modified the standard hemiparkinsonian rat model. The changes we made allow destruction of the substantia nigra while sparing the ventral tegmental area (VTA) (this model is analogous to the MPTP hemiparkinsonian monkey model). In this model, there is some residual tyrosine hydroxylase activity in the medial portion of the caudate, but very consistent and stable turning occurs in response to amphetamine. Recently we established behavioral criteria which are based on amphetamine and L-Dopa -induced turning, and which permits us to select rats into appropriate experimental groups based on the intactness of the innervation of the caudate from the VTA. We are examining cellular implants, delivered stereotactically, and chemicals, delivered by polymer implantation and by direct infusions, to assess the influence on the turning behavior and induction of neuronal sprouting. Three cell populations have been examined in this model. Activated inflammatory cells from the peritoneal cavity of the rat implanted into the denervated caudate lead to significant motor improvement, which is not seen in sham-implanted or inactivated cell-implanted hemiparkinsonian rats. There is evidence that the improvement is mediated through an intrinsic dopaminergic mechanism. Implantation of cultured rat neonatal microglial cells also leads to improvement in this model. Finally, cell suspension grafts of amnion lead to moderate recovery, which is not seen when killed amnion cells are implanted. The most dramatic results were obtained after implantation of IL-1 polymer pellets. The results with inflammatory cells, microglia, and IL-1 may reflect a cytokine/glia/neuronal interaction which leads to increased turnover of dopamine or to sprouting of new dopaminergic fibers.

C. Molecular Biology Unit

The Molecular Biology Unit of the Surgical Neurology Branch is studying the genetic abnormalities of various brain disorders, especially brain tumors. During the past 2 years we have focused on molecular genetic analyses of two CNS tumors, glioblastomas and pituitary adenomas.

Glioblastomas

Extensive studies of the molecular origins of various human cancers done over the past several years have implicated mutational activation of dominantly acting proto-oncogenes in various malignancies. Recently, yet another mechanism,

homozygous loss of gene function, is believed to be one of the genetic events involved in the development of certain neoplasms.

Glial tumors offer an excellent model system to study molecular mechanisms that confer upon these cells an increased ability to grow, interact with the environment, and eventually metastasize. A variety of genetic lesions may be expected to contribute to the expression of the neoplastic phenotype of gliomas. We have taken the following approaches to understand the genetic aberrations responsible for the expression of malignant phenotype in glial tumors, which probably evolve through a series of genetic lesions.

1. Autocrine loops of the growth signaling pathways involving platelet-derived growth factor receptors (PDGFR) and epidermal growth factor receptors (EGFR) and their ligands have been implicated in the etiology of gliomas. We have analyzed the genomic organization and expression of PDGFRs and EGFR in 50 glial tumors. Amplification and/or overexpression of the α form of PDGFR and EGFR was detected in distinct subsets of glial tumors. Furthermore, most of the tumors with elevated levels of α PDGFR also overexpressed β PDGFR, which was not amplified at the gene level in any of the 50 tumors analyzed.

Our previous analysis did not show amplification and/or gross rearrangements of various growth factors and growth factor receptors genes in primary glial tumors or cell lines derived from glial tumors. However, a generalized elevation of basic fibroblast growth factor, its high affinity receptor, flg, and transforming growth factors α and β , was detected in most of the glioblastoma cell lines examined.

2. The loss of heterozygosity of specific genes, as detected by restriction fragment length polymorphism analysis, suggests the presence of tumor suppressor genes in the deleted areas of the chromosome. We have so far analyzed glial tumors and their matched lymphocytes from 40 patients for possible allelic deletions of genes on various chromosomes and have identified loss of heterozygosity of genes on chromosomes 10 and 17 in a significant number of tumors. Most of the tumors with deletions on chromosome 17 include the p53 gene, whose homozygous inactivation by deletion of one allele and mutation in the remaining allele represents the most common genetic alteration in human cancers. In our panel of tumors, such homozygous inactivation of the p53 gene was not always observed in tumors with gene losses on chromosome 17. Furthermore, deletion mapping analysis suggested the presence of at least one other tumor suppressor gene in the telomeric region of the short arm of chromosome 17.
3. There are no well-defined clinical or biologic differences between primary and recurrent glioblastomas. Recurrent tumors, however, are generally considered to be less well differentiated and sometimes more aggressive. We have analyzed six pairs of primary and recurrent tumors from the same patients for the loss of heterozygosity of genes on several chromosomes. Our data clearly provide evidence for additional genetic abnormalities including mutation in the p53 gene in recurrent glioblastomas.

Pituitary Adenomas

Corticotroph adenomas are ACTH (adrenocorticotrophic hormone)-producing tumors that originate in the corticotrophs of the pituitary gland. These tumors cause increased ACTH secretion and consequent hypersecretion of cortisol. Corticotroph adenomas in association with Cushing's disease are often microadenomas which do not grow over several years. However, in some patients, with a condition called Nelson's syndrome, adrenalectomy gives rise to hypersecretion of ACTH by pituitary adenomas which are usually large, aggressive, and rapidly growing neoplasms. Hypersecretion of ACTH can also be caused by ectopic nonpituitary tumors.

Our previous results demonstrated that pituitary corticotroph adenomas can be monoclonal or polyclonal, thus emphasizing the complexity of genetic events with fundamentally different mechanisms involved in oncogenesis. We have therefore taken multiple approaches to study different kind of ACTH-secreting adenomas.

Mutations leading to the activation of the transforming potential of cellular ras genes have been implicated in the development of many human tumors of diverse origin. Our analysis of PCR amplified hot spot regions of all three ras regions in 27 pituitary adenomas followed by allele-specific oligonucleotide hybridization indicated that ras mutations are very rare in corticotroph adenomas.

Amplification followed by sequencing of the POMC promoter region from various types of 11 ACTH-producing tumors demonstrated that there are no genetic defects in the POMC transcriptional regulatory region. This suggests that the defective glucocorticoid suppression may be due to the abnormalities either in the hormone receptor or in other compounds of the transcriptional regulatory mechanism.

Initial analysis showing allelic loss of genes on chromosome 17 suggests that the p53 gene may have a role in the progression of aggressive corticotroph tumors.

Structural Analysis of Gs

Alpha subunits of G proteins play a central role in signal transduction between the membrane-bound hormone receptors and neurotransmitters and intracellular enzymes and other effectors. Somatic mutations, which inhibit the GTPase activity of the stimulatory (Gs) or inhibitory (Gi2) subunits of G proteins, thereby rendering them potentially oncogenic, have been detected in certain endocrine tumors. It is also possible that other forms of the alpha subunit of G proteins and/or other mechanisms of activation other than point mutations are involved. We have identified aberrantly spliced truncated Gs α transcripts in transformed human astroglial and glioblastoma cell lines. Consistent with this, smaller size Gs α peptides were also detected in these cell line. Also, our initial analysis showed the presence of aberrant Gs α transcripts in the CNS tumors. The biological relevance of abnormal Gs α splicing remains to be determined.

II. BIOCHEMISTRY SECTION

Richard J. Youle, Ph.D., Chief

Monoclonal Antibody-Mediated Killing of Tumor Cells

The Section of Biochemistry is studying the use of monoclonal antibodies to kill disease-causing cells. Monoclonal antibodies which selectively bind tumor cells can be generated, but alone are usually not cytotoxic to the tumor. Toxic proteins such as ricin and diphtheria toxin (DT) can be chemically linked to monoclonal antibodies. The new hybrid molecules bind tumor cells via the antibody moiety and then kill the cells via the toxin moiety. The toxins used are enzymes that catalytically inactivate protein synthesis in target cells with only one or two molecules in the cytoplasm killing a cell. A major goal of the laboratory is to develop immunotoxins which will selectively kill tumor cells in vivo. Currently, the limiting steps for antibody-toxin hybrids are (1) the entry of the toxin molecule into the cell; (2) in vivo access of the drug to the tumor cells; and (3) immunogenicity of the foreign proteins.

The discovery of point mutants of DT that increase immunotoxin selectivity in FY88 and the demonstration of immunotoxin activity in animal models of leptomeningeal cancer in FY89 were prepared for clinical trials in FY90 and 91. Extensive toxicity testing has shown that a 100- to 1000-fold therapeutic window exists between the concentration toxic to tumor cells and the dose tolerable in rats, guinea pigs and monkeys. Based upon our thorough preclinical testing, we determined that immunotoxins may offer a therapeutic benefit to patients with leptomeningeal carcinomatosis and medulloblastoma. We have begun clinical trials with a monoclonal antibody against the human transferrin receptor linked to recombinant ricin A chain supplied by Cetus Corp. We have treated 8 patients so far in a dose escalation that has spanned a 1000-fold increase in amount of drug injected. We appear to be close to the dose-limiting toxicity. At this stage we cannot establish efficacy of the drug to the patients. However, significant decreases in CSF tumor cell counts were achieved in 4 of 7 patients. We have also begun direct infusion of Tfu-FRM107 into patients with brain tumors. We will continue our studies with this reagent as well as prepare second-generation immunotoxins that may offer greater potency for clinical trials.

To improve entry of immunotoxins into cells, we have modified toxins chemically and genetically to make deletion and point mutations. We are studying the structure-function relations of the toxin molecule and the cell biology of toxin internalization and membrane penetration. We have identified point mutations in the translocation region of DT that block toxicity up to 1000 times. Proline 345 in the DT B chain appears to play an essential role in the transmembrane transport.

We have found ways to block the immune response animals generate against immunotoxins. Injection of a monoclonal antibody against the helper T-cell CD4 antigen completely prevent the primary immune response to weekly injections of immunotoxins for 3 months or more.

Toxic proteins from bacteria and plants inactivate protein synthesis enzymatically. Analogous toxic mammalian proteins have not been described but some members of the ribonuclease superfamily in mammals have interesting, possibly related, biological properties. A human serum ribonuclease (angiogenin) was shown to abolish cell-free protein synthesis by inactivating the small ribosomal

subunit of rabbit reticulocyte ribosomes and cytotoxic eosinophil granule proteins also have been reported to have RNase activity. The sequence of human eosinophil-derived neurotoxin (EDN) is identical to that of the nonsecretory ribonuclease from human urine and homologous to pancreatic RNase. We found that RNase, delivered to cells via the transferrin receptor-mediated endocytosis pathway would be toxic to cells. RNase may be used to specifically kill target cells via cell surface receptors or antigens when it is chemically linked or genetically fused to a cell binding moiety. Thus, these human proteins, that may be much less immunogenic than plant or bacterial toxins, may be useful as immunotoxins.

During investigation of the clinical potential for immunotoxins injected intrathecally we noticed that animals became ataxic. Thorough analysis of this revealed that immunotoxins specifically kill up to 70% of Purkinje cells in animals with no detectable toxicity to other neurons or cells. This affords a new animal model of Purkinje cell loss, yields a valuable clue to the potential dose limiting toxicity of immunotoxins in clinical trials, and points to a new receptor-mediated uptake pathway in the CNS.

Programmed Cell Death in the Nervous and Immune Systems

Our laboratory has begun a project to study the mechanism and physiologic role of programmed cell death. In both the nervous and immune systems, massive numbers of cells die during normal development. In the spinal cord, for example, over half the neurons die in some nuclei during fetal development. The immune system also has large cell loss at precise stages in development such as thymocyte death and thymus involution at puberty. The physiologic role and the biochemical mechanism of programmed cell deaths are unknown. Understanding the mechanism and regulation of such physiologic cell deaths may shed light on neurodegenerative diseases and immunodeficiency disorders.

We have found that thymocyte-programmed cell death can be followed morphologically with Normarski optics, and that the thymocyte death resembles neuronal cell death. The morphologic analysis of nuclear disintegration has allowed us to test whether cell death is due to production of a toxic factor or due to the loss of a protective factor. Using the new microscopic method to identify apophysis, the nuclei in the heterokaryons were found to follow the original and distinct fate of the parent cells and not to transfer apoptosis nor viability between nuclei.

We have found that MPP⁺ induces apophysis in cerebellar granule cells in vitro, and we are examining the mechanism of granule toxicity on these cells. We have also discovered a new, in situ method to measure the DNA strand breaks of apophysis. This will allow us to examine animal and human brain tissues and probe the mechanism of cell death during normal development and during neurodegenerative diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02850 - 01 SN

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Therapy for Brain Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D.

Chief, SNB, NINDS

Zvi Ram, M.D.

Visiting Scientist, SNB, NINDS

Stuart Walbridge

Biologist, SNB, NINDS

Kenneth Culver, M.D.

Senior Clinical Investigator, MB, NCI

R. Michael Blaese, M.D.

Chief, Cellular Immunology, MB, NCI

COOPERATING UNITS (if any)

National Cancer Institute, Bethesda, Maryland

Genetic Therapy, Gaithersburg, Maryland

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We investigated the possibility of selective in vivo transduction of growing brain tumors with retroviral vectors carrying the herpes simplex thymidine kinase (HS-tk) gene to confer drug sensitivity to the anti-viral drug ganciclovir (GCV). We have shown that rats with a malignant brain tumor which were given an intratumoral stereotaxic injection of murine fibroblasts that were producing the HS-tk vector and then treated with GCV had regression of their tumors. Rats treated with a control vector producer cell line containing the beta-galactosidase gene (lacZ gene) developed large tumors and died. Using the lacZ gene as a reporter gene, we were able to study the dynamics of in situ tumor transduction in rats. Dose-response studies of GCV and various concentrations of the injected vector-producer cells provided further data to guide us in designing a clinical protocol for the treatment of patients with brain tumors. No significant toxicity was observed in toxicity studies in mice, rats, and nonhuman primates.

Long-term survival experiments using this approach showed a cure rate of 30-35% and significant prolongation of survival in the remainder of the treated rats. Survival curves suggest the need for repeat treatment to enhance the efficacy of our approach.

Based upon these findings, we have proposed a human clinical trial to determine the efficacy of HS-tk transduction in patients with brain tumors.

Retroviral-mediated gene transfer into experimental brain tumors using cytokine genes (IL-2), alone or as a combined HS-tk/IL-2 vector, were also evaluated. However, no enhancement of tumor eradication was observed with the addition of the cytokine gene confirming the limited usefulness of immune enhancement within the CNS.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02868-01 SN

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Semi-Chronic Intracortical Electrical Stimulation of the Visual Cortex of a Blind Volunteer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Conrad Kufta, M.D.

Medical Officer, SNB, NINDS

Daniel O'Rourke, M.D.

Clinical Associate, SNB, NINDS

Martin Back

Electrical Engineer, LNLC, NINDS

Edward Schmidt, Ph.D.

Biological Engineer, LNLC, NINDS

F. Terry Hambrecht, M.D.

Head, Neuroprothesis, NINDS

COOPERATING UNITS (if any)

Howard Hughes Fellow - P. Vallabhanath

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to evaluate the feasibility of a visual prosthesis for totally blind individuals by stimulating chronically implanted microelectrodes in the visual cortex. A 42 year-old woman who has been blind for 22 years was implanted with an array of 38 electrodes in the visual cortex. Stimulation of individual electrodes produced sensation of light called phosphenes. Phosphenes were produced with 34 of the 38 electrodes with currents that were 100 to 1000 times lower than had been reported for surface stimulation of the visual cortex. Additional blind patients need to be tested before we will know if intracortical microstimulation (ICMS) of the visual cortex is a feasible technique for producing a visual prosthesis. However, all the tests performed to date indicate that ICMS may be feasible.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS02855-01 SN
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Interstitial Therapy with Targeted Protein Toxins for Malignant Brain Tumors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> PI: Edward H. Oldfield, M.D. Douglas W. Laske, M.D. Richard J. Youle, Ph.D. Orhan Ilcercil, M.D. David Katz, M.D. Nicholas Patronas, M.D. </div> <div style="width: 45%;"> Chief, SNB, NINDS Senior Staff Fellow, SNB, NINDS Chief, Biochemistry Section, SNB, NINDS Clinical Associate, SNB, NINDS Neuropathologist, OD, NINDS Radiologist, CC </div> </div>		
COOPERATING UNITS (if any) Department of Radiology, CC		
LAB/BRANCH Surgical Neurology Branch, NINDS		
SECTION Clinical Neurosurgery Section, SNB, NINDS		
INSTITUTE AND LOCATION NINDS, National Institutes of Health, NINDS		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>We are investigating a new experimental approach for the treatment of recurrent <u>malignant brain tumors</u> which utilizes a new class of anti-cancer compounds, called <u>targeted protein toxins</u>. A targeted protein toxin is a compound that consists of two parts. The binding moiety targets the conjugate to the surface of tumor cells bearing the appropriate antigen or receptor and the toxin moiety then penetrates the cell membrane and inactivates protein synthesis, resulting in cell death.</p> <p>The specific compound under investigation in this trial, <u>transferrin-CRM107</u> (Tf-CRM107), is a conjugate of human transferring (Tf) and diphtheria toxin (DT) with a point mutation (CRM107). The binding moiety, Tf, binds to the transferrin receptor which helps cells take up iron and is present in higher number on tumor cells than on normal brain cells. The complete compound, Tf-CRM107, can kill tumor cells preferentially at low concentration, and when directly injected into tumors, causes tumor shrinkage in animal models of primary human malignant brain tumors.</p> <p>We have initiated a phase I trial of regional therapy with Tf-CRM107 treat recurrent malignant brain tumors. In this clinical trial, Tf-CRM107 will be delivered by slow intratumoral and peritumoral infusion. The purpose of this study is to determine if this new experimental compound, Tf-CRM107, can be used safely in patients with malignant brain tumors. We also hope to learn about its ability to kill tumor cells in patients with malignant brain tumors.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02859-015N

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Programmed Cell Death in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard J. Youle, Ph.D.

Chief, Biochemistry Section, SNB, NINDS

Bruno Dipasquale, M.D.

Visiting Associate

Katherine A. Wood, Ph.D.

Visiting Fellow

COOPERATING UNITS (if any)

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

3

PROFESSIONAL:

3.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have begun to study programmed cell death in the nervous system and the biochemical mechanism of apoptosis in general. To approach the nervous system more sensitive and in situ methods are needed to identify cells undergoing programmed cell death. We have developed two new methods to identify apoptotic cells under the microscope. 1) We have found that thymocyte programmed cell death can be followed morphologically with Nomarski optics and that the thymocyte death resembles neuronal cell death. The morphologic analysis of nuclear disintegration has allowed us to test whether cell death is due to production of a toxic factor or due to the loss of a protective factor. Using the new microscopic method to identify apoptosis, the nuclei in the heterokaryons were found to follow the original and distinct fate of the parent cells and not to transfer apoptosis nor viability between nuclei. This new method also allowed us to identify apoptosis as the method of cerebellar granule cell death after MPP⁺ treatment in vitro. 2) We have also developed a molecular detection method to measure DNA strand breaks in situ. This allows us to examine brains of animals undergoing neurodegenerative changes during ischemia, MPTP treatment, and during development. This new method should illuminate the role apoptosis plays during development and during various disease states of the nervous system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02854 - 01 SN
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Establishing the Physiology of Syringomyelia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Edward H. Oldfield, M.D. John D. Heiss, M.D., Senior Staff Fellow, SNB Charles Haworth, M.D., LCDR, MC, USNR Thomas Shawker, M.D., CC Radiology William Kammerer, M.D., CC Anesthesiology Thomas Talbot, RR, BEIP Thomas Clem, RR, BEIP	Chief, SNB, NINDS Morris Pulliam, M.D., Capt, MC, USN Nick Patronas, M.D., CC, Radiology Robert Dedrick, Ph.D., RR, BEIP Alec Eidsath, Ph.D., RR, BEIP Elijah Walker, RR, BEIP	
COOPERATING UNITS (if any) Diagnostic Radiology Department, CC Anesthesiology Department, CC RR, BEIP		
LAB/BRANCH Surgical Neurology Branch, NINDS		
SECTION Clinical Neurosurgery Section, SNB, NINDS		
INSTITUTE AND LOCATION NINDS, National Institutes of Health, NINDS		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The purpose of this study is to establish the mechanism(s) of progression of communicating syringomyelia. Communicating syringomyelia usually accompanies abnormalities at the craniocervical junction. Measurement of intraventricular pressure, intrathecal pressure, and intrasyrninx pressure should provide data which elucidate the hydrodynamic mechanism(s) of progression of syringomyelia. Radiographic testing, including MRI flow studies, ultrasonography, and Imatron CT, will demonstrate how pathologic anatomy alters normal cerebrospinal fluid (CSF) flow. The effect of posterior fossa craniectomy, upper cervical laminectomy, and duraplasty on CSF flow, syrinx size, and neurologic function will be evaluated.</p> <p>One patient has been treated to date. The pressure measurements were performed without complication. CSF circulation at the foramen magnum improved with surgery in this patient. The syrinx decreased in size following surgery. We plan to proceed with an additional nine patients.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02840-02 SN

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Alpha Subunits of G Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Iqbal Ali, Ph.D.

SNB, NINDS

Willial Reinhold

Chemist, SNB, NINDS

Edward H. Oldfield, M.D.

Chief, SNB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Molecular Biology Unit

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Components of signaling pathways that promote proliferation are likely to play a central role in normal cellular growth and differentiation, and are therefore potential targets during pathogenesis, especially neoplastic growth. Guanine nucleotide binding (G) proteins are membrane-associated heterotrimers (alpha, beta, and gamma subunits) and play an important role in transmembrane signal transduction. One type of alpha subunit, Gs, is ADP-ribosylated by cholera toxin and mediates activation of adenylate cyclase. Two point mutations, found at the cholera toxin ribosylation site and a proposed confirmational switching area (S-box), have been proposed to be oncogenic in a subset of growth hormone-producing pituitary adenomas.

We have carried out G-specific PCR amplification and subsequent cloning of amplified cDNAs from normal human brain tissue, placenta, an SV40-transformed human astroglial cell line, a glioblastoma cell line, (H5683) a primary glioblastoma, and an ACTH-producing pituitary adenoma. Characterization of the recombinant clones showed the presence of novel truncated Gs transcripts in the transformed astroglial cell line SVG, glioblastoma cell line H5683, and glial and corticotroph tumors. Our results suggest that aberrant splicing of Gs may have a modulatory function in transformation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02814-03 SN

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Abnormalities in Primary Glial Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Iqbal U. Ali, Ph D.	SNB, NINDS		
Abha Saxena	Visiting Associate, SNB, NINDS	Timothy Fleming,	NCI
Barbara Ikejiri	Biologist, SNB, NINDS	Stuart Aaronson,	Chief, LCMB, NCI
Richard Berkman, M.D.	Clinical Associate, SNB, NINDS		
Edward H. Oldfield, M.D.	Chief, SNB, NINDS		
WC Clark, M.D.	Head, Section of NS, University of Tennessee		
James Robertson, M.D.	Chairman, Dept. of NS, University of Tennessee		

COOPERATING UNITS (if any)

University of Tennessee, Memphis, Tennessee
LCMB, NCI

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Molecular Biology Unit, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gliomas are the most common primary brain tumors ranging from benign low-grade astrocytomas to highly malignant glioblastoma multiforme. The classification of primary glial tumors reflecting their biological aggressiveness is based upon histopathologic characteristics. We have taken several approaches to understand and identify, at a molecular level, the underlying mechanisms that translate into the extremely malignant behavior of glioblastoma.

1. We have used X-chromosome inactivation analysis to study the clonal composition of glioblastomas, which were found to be monoclonal.
2. The genomic organization and expression of PDGFRs and EGFR were analyzed in 50 glial tumors. α -PDGFR and EGFR were amplified, whereas α - and β -PDGFR and EGFR were overexpressed in distinct subsets of glial tumors.
3. The transcription of bGFG, its surface-associated receptor, flg, TGF α , and TGF β , was generally elevated in most of the glioblastoma cell lines tested.
4. Recessive mutations that predispose to cancer are unmasked in several human cancers by the loss of normal alleles. Restriction fragment length polymorphism analysis (RFLP) was used to compare the constitutional and tumor genotypes in a panel of 40 glioblastomas. Loss of heterozygosity of several markers on chromosomes 17 and 10 was detected in a significant number of glioblastoma multiforme. The p53 gene was deleted and/or mutated in 75% of the tumors with gene losses on chromosome 17. Deletion mapping studies on chromosome 17 suggested the presence of another potential tumor suppressor gene distinct from the p53 gene.
5. Six matched pairs of primary and recurrent tumors were analyzed for allelic deletions of chromosomes 10 and 17. The data clearly demonstrated additional genetic abnormalities in recurrent tumors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02813-03 SN
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pharmacokinetics of Direct Brain Infusion		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Edward H. Oldfield, M.D. Douglas W. Laske, M.D. Orhan Ilcili, M.D. Aytac Akbasak, M.D. Bob Boock, Ph.D. Paul Morrison, Ph.D. Robert Dedrick, Ph.D.	Chief, SNB, NINDS Senior Staff Fellow, SNB, NINDS Clinical Associate, SNB, NINDS Visiting Associate, NINDS Senior Staff Fellow, SNB, NINDS Biomedical Engineering, RR Biomedical Engineering, RR	
COOPERATING UNITS (if any)		
LAB/BRANCH Surgical Neurology Branch, NINDS		
SECTION Clinical Neurosurgery Section, SNB, NINDS		
INSTITUTE AND LOCATION NINDS, National Institutes of Health, NINDS		
TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>For many compounds (neurotrophic factors, antibodies, growth factors, genetic vectors, enzymes) minimal diffusion in the brain severely limits drug distribution after direct drug administration in to brain parenchyma. We systemically investigated convection, molecular transport with bulk flow of fluid, to enhance the distribution of large and small molecules, indium¹¹¹-transferrin (In¹¹¹-Tf; MW 80,000) and C¹⁴-sucrose (MW 359), by maintaining a pressure gradient during interstitial infusion to generate bulk flow through the brain interstitium. The volume of distribution (V_d) containing ≥ 1% of infusate concentration increased linearly with the infusion volume (V_i) for In¹¹¹-Tf (V_d/V_i = 6.1) and C¹⁴-sucrose (V_d/V_i = 14.1). 24 hr after infusion, the distribution of In¹¹¹-Tf increased, became more homogeneous, and penetration into gray matter occurred. By using convection to supplement simple diffusion, greatly enhanced distribution of large and small molecules can be achieved in the brain while achieving drug exposure orders of magnitude greater than systemic exposure.</p>		

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 NS 02812-03 SN

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pentobarbital Effects on Damage of the Primate Brain by Fractionated Whole Brain Radiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D.

Chief, SNB, NINDS

Aytac Akbasak, M.D.

Visiting Associate, SNB, NINDS

Tom Goffman, M.D.

Radiation Oncology Branch, NCI

Kathryn Orr, R.N.

Radiation Oncology Branch, NCI

Calvin Hawkins

Bio Lab Technician, SNB, NINDS

Lisa Berney

LATG

COOPERATING UNITS (if any)

Radiation Oncology Branch, NCI

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Radiation therapy remains the single most effective treatment for malignant brain tumors, but in many cases, toxicity to normal brain impedes therapeutic doses sufficient for local control to be achieved. A substantial effort has been directed toward overcoming the unfavorable side effects of brain tumor radiation therapy.

Data from our institute and other indicate the concomitant application of pentobarbital anesthesia during cerebral irradiation reduces the toxicity of the ionizing radiation. Although mechanisms of this phenomenon remains unclear, it seems to arise from general suppression of brain synaptic activity or metabolism.

After baseline MRI scans of the brain and neuroendocrine testings, primates (*Macaca mulatta*) undergo whole brain X-irradiation in 10 daily fractions, 360 rads each, total dose of 3600 rads. The monkeys in the study group were anesthetized with pentobarbital during the irradiation whereas the animals in the control group received ketamine. Each group consists of six animals. Neuroendocrine testing and MRI scan follow-up studies are performed at 3, 6, 12, 18 and 24 months after irradiation. Quantitative histology will be done on the capillary bed, glial and neuronal populations after sacrifice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02815-03 SN

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Pituitary Corticotroph Adenomas

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Iqbal Ali, Ph.D.

SNB, NINDS

Heiner Monig

Visiting Associate, SNB, NINDS

David Katz, M.D.

Neuropathologist, OD, NINDS

Barbara Ikejiri

Biologist, SNB, NINDS

Edward Oldfield, M.D.

Chief, SNB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Molecular Biology Unit

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cushing's disease is caused by the pituitary hypersecretion of ACTH and occurs predominately in women. Patients are cured by surgical removal of an ACTH-producing adenoma, suggesting evolution and expansion of a genetically aberrant cell. However, hypothalamic dysfunction and excessive stimulation of anterior pituitary corticotrophs by one or more neurotransmitter substances may also lead to the development of corticotrophic adenomas.

To study the clonal composition of ACTH-producing pituitary adenomas, we used restriction fragment length polymorphism (RFLP) of two X-chromosome linked genes, hypoxanthine phosphoribosyl transferase (HPRT) and phosphoglycerate kinase (PGK), in conjunction with their methylation patterns. Analysis of 27 tumors demonstrated a monoclonal pattern in six of these tumors, whereas a polyclonal pattern was observed in three tumors including a pituitary adenoma from a patient with the Nelson's syndrome.

Amplification by the polymerase chain reaction, (PCR), of the mutational hot spots in ras genes followed by allele specific oligonucleotide (ASO), hybridization showed mutation in one of the 27 tumors. We also searched for mutations in the promoter region of the POMC gene by PCR amplification and sequencing. This region was found to be perfectly normal in 11 tumors.

Allelotyping of the pituitary tumors is being carried out by using restriction fragment length polymorphism (RFLP) analysis. Initial studies showed loss of heterozygosity of genes on chromosome 17 in one of the 4 Nelson's tumors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02823-03 SN														
PERIOD COVERED October 1, 1991 - September 30, 1992																
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Antibody-Toxin Conjugates for the Treatment of Human Brain Tumors																
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 35%;">PI: Richard J. Youle, Ph.D.</td> <td>Chief, Biochemistry Section, SNB, NINDS</td> </tr> <tr> <td>Doug Laske, M.D.</td> <td>Senior Staff Fellow, SNB, NINDS</td> </tr> <tr> <td>Orhan Ilıcil, M.D.</td> <td>Clinical Associate, SNB, NINDS</td> </tr> <tr> <td>Edward H. Oldfield, M.D.</td> <td>Chief, SNB, NINDS</td> </tr> <tr> <td>David Katz, M.D.</td> <td>Neuropathologist, OD, NINDS</td> </tr> <tr> <td>Cynthia Sung, Ph.D.</td> <td>Staff Fellow, PEIB</td> </tr> <tr> <td>Robert Dedrick, Ph.D.</td> <td>Senior Staff Fellow, PEIB</td> </tr> </table>			PI: Richard J. Youle, Ph.D.	Chief, Biochemistry Section, SNB, NINDS	Doug Laske, M.D.	Senior Staff Fellow, SNB, NINDS	Orhan Ilıcil, M.D.	Clinical Associate, SNB, NINDS	Edward H. Oldfield, M.D.	Chief, SNB, NINDS	David Katz, M.D.	Neuropathologist, OD, NINDS	Cynthia Sung, Ph.D.	Staff Fellow, PEIB	Robert Dedrick, Ph.D.	Senior Staff Fellow, PEIB
PI: Richard J. Youle, Ph.D.	Chief, Biochemistry Section, SNB, NINDS															
Doug Laske, M.D.	Senior Staff Fellow, SNB, NINDS															
Orhan Ilıcil, M.D.	Clinical Associate, SNB, NINDS															
Edward H. Oldfield, M.D.	Chief, SNB, NINDS															
David Katz, M.D.	Neuropathologist, OD, NINDS															
Cynthia Sung, Ph.D.	Staff Fellow, PEIB															
Robert Dedrick, Ph.D.	Senior Staff Fellow, PEIB															
COOPERATING UNITS (if any) Diagnostic Radiology; Nuclear Medicine Department; National Cancer Institute, Hafslund Nycomed																
LAB/BRANCH Surgical Neurology Branch, NINDS																
SECTION Biochemistry Section																
INSTITUTE AND LOCATION NINDS, National Institutes of Health, NINDS																
TOTAL STAFF YEARS: 2.5	PROFESSIONAL: 2.5	OTHER: 0.0														
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input checked="" type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews							
<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither														
<input type="checkbox"/> (a1) Minors																
<input type="checkbox"/> (a2) Interviews																
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A phase I dose-escalation study of intrathecal therapy with the immunotoxin 45A12-RTA for leptomeningeal neoplasia has been completed. This compound is a conjugate of a monoclonal antibody against the human transferrin receptor and the recombinant ricin A chain protein toxin. Eight patients with leptomeningeal spread of systemic neoplasia were treated with a total of ten different doses of intrathecal immunotoxin covering a 1000-fold increase in drug dose (1.2 to 1200 micrograms).</p> <p>No toxicity was detected until the highest doses were reached. Acute toxicity consisted of transient headache, vomiting and decreased mental status with elevated intracranial pressure which was responsive to steroids and CSF drainage. Bioassays of serial CSF samples from these patients against tumor cell lines in vitro revealed that patient's CSF retained cytotoxic activity against tumor cells for approximately 48 hours after intraventricular administration of immunotoxin. In addition, in vitro testing of 45A12-RTA against tumor cells harvested from the spinal fluid in 3 study patients revealed tumor cell sensitivity to the drug before and after treatment at concentrations of drug much lower than the concentration achieved in CSF. Four patients had decreased lumbar CSF tumor cell counts, the most dramatic (>95%) occurring at the highest dose given.</p> <p>These results indicate that immunotoxin can be safely administered intrathecally in humans, retain bioactivity in the CSF, are cytotoxic to tumor cells from patients, and can reduce tumor burden after only a single dose.</p> <p>A new clinical trial of a genetically engineered immunotoxin, Tfn-CRM107, discovered with the branch has begun for treatment of parenchymal brain tumors.</p>																

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02781-05 SN

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tissue Implantation in Parkinsonian Models

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D.

Chief, SNB, NINDS

Kris Bankiewicz, M.D.

Visiting Scientist, SNB, NINDS

Daniel Lieberman, M.D.

Staff Fellow, SNB, NINDS

Alex Cummins, M.S.

Biologist, SNB

Hicleki Takubo, M.D.

Visiting Associate, NINDS

Martha Johnson

Histopathology Technician

COOPERATING UNITS (if any)

David Jacobowitz, Clinical Neuropharmacology, NIMH, Charles Gerfen, Neurophysiology, NIMH, Ivan Mefford, Neurochemistry, NIMH

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

CNS Transplantation Unit

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

6.5

PROFESSIONAL:

6.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The behavioral, biochemical, and histologic effects of tissue implants in rodent and primate models of parkinsonism is being studied. The grafts which have been examined include fetal and adult dopaminergic and nondopaminergic tissues. There is some behavioral improvement with any operative trauma to the caudate, whether or not a graft is placed. Generally, fetal tissue grafts (dopaminergic or nondopaminergic) lead to a much greater degree of recovery than adult tissue grafts or trauma alone. The histologic observation of dopaminergic fiber ingrowth (sprouting) in all these animals suggests that the improvement is mediated through a neurotrophic interaction. We are trying to determine the cell-to-cell interaction which leads to new growth of fibers from an adult neuron, using in vivo and in vitro methods. Two major areas of emphasis are: what cascade of events in the host after trauma leads to sprouting and why does fetal tissue enhance the recovery (even nondopaminergic tissue).

To identify areas of the brain that are involved in host dopaminergic sprouting a series of experiments injecting anterograde and/or retrograde tracers were performed. The dopaminergic cells in the ventral tegmental area and the periaqueductal area seems to be involved in the sprouting response.

Another area of investigation addresses the question of how similar is the dopaminergic cell loss in the midbrain of idiopathic parkinsonian patients to cell loss in the monkeys treated with MPTP. We were able to identify anatomic subregions in the midbrain of parkinsonian patients still containing the dopaminergic cells. These areas are almost identical to the areas that are preserved in parkinsonian monkeys, suggesting that indeed there are dopaminergic cells in the parkinsonian brains that may be a source for the graft-induced host sprouting.

The experiments included implantation of fetal and term amnion into hemiparkinsonian monkeys (solid tissue into preformed cavities), cell suspension implants of term amnion into rats, IL-1 slow-release polymers implants in hemiparkinsonian rats and monkeys. To further investigate the host response to tissue implantation, we are now using direct intrastriatal infusion of chemicals known to be released by inflammatory cells. Effects of direct intrastriatal infusion of trophic factors as basic FGF, IGF-1, laminin, PDGF and IL-1 on the host dopaminergic system is now being studied in hemiparkinsonian rats. These animals displayed histologic signs of sprouting from the remaining dopaminergic neurons. Experiments on delivering trophic factors into hemiparkinsonian monkeys is underway.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02739-06 SN

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Laboratory Investigation of Central Nervous System Vascular Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D.,	Chief, SNB, NINDS
Ryszard Pluta, M.D.	Visiting Associate, SNB, NINDS
Robert Boock, Ph.D.	Staff Fellow, SNB, NINDS
John Abshar, M.D.	Clinical Associate, SNB, NINDS
Marston Linehan, M.D.	Surgical Branch, NCI
Berton Zbar, M.D.	Senior Investigator, NCI
Gregory Thompson, M.D.	Clinical Associate, SNB, NINDS

COOPERATING UNITS (if any)

Diagnostic Radiology Department, CC, Experimental Therapeutics Branch, NINDS
Surgery Branch, National Cancer Institute

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients with Von Hippel-Lindau disease were investigated and the following were shown: investigation of the molecular biology of hemangioblastomas of the central nervous system (CNS) revealed an homozygous deletion of a portion of the short arm of the third chromosome, demonstrating that these tumors are probably caused by the absence of a tumor-suppressing gene, as are familial retinoblastomas. MRI with gadolinium-EDTA contrast enhancement was shown to be a sensitive technique for detection of small hemangioblastomas of the CNS. Excision of the tumors alone was shown to result in resolution of syringomyelia associated with spinal cord hemangioblastomas. Therefore, the tumor-associated syrinx does not need separate treatment.

Endothelial- derived relaxation factor (nitric-oxide) was shown to mediate autoregulation and chemoregulation of cerebral blood flow. Nitricoxide synthase immunoreactivity was demonstrated in the nerve plexus in the adventitia of the Circle of Willis in primates.

During cerebral vasospasm in primates, disappearance of immunostaining for nitroxide synthase from the adventitial plexus of the involved artery was demonstrated. Immunoreactivity returned after resolution of cerebral vasospasm. Cerebral blood flow in the distribution of cerebral vasospasm could be increased by intracarotid infusion of L-arginine, the substrate for nitric oxide production. These findings suggest that regulation of nitric oxide production is impaired in cerebral vasospasm and that this impairment may underlie the etiology of cerebral vasospasm.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02708-07 SN

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vascular Permeability Factor Produced by Human Glioma Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Marsha Merrill, Ph.D. Senior Staff Fellow, SNB, NINDS
 Richard Berkman, M.D. Clinical Associate, SNB, NINDS
 John Heiss, M.D. Senior Staff Fellow, SNB, NINDS
 Mima Bacic, M.D., Ph.D. Visiting Associate, SNB, NINDS
 Nancy Edwards, B.A. Biologist, SNB, NINDS
 Calvin Hawkins Bio Lab Technician, SNB, NINDS
 Edward H. Oldfield, M.D. Chief, SNB, NINDS

COOPERATING UNITS (if any)

Walter Reed Army Medical Center
 Molecular Biology Unit, SNB, Iqbal Ali, Ph.D.

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Tumor Biology Unit

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

3.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF), has been proposed to be a mediator of endothelial proliferation and angiogenesis in normal and diseased states, and to have a role in the development of tumor-associated vascular hyperpermeability. The purpose of this study was to examine expression of the VPF/VEGF gene in both tumor and normal tissues.

In a study of the levels of VPF/VEGF mRNA in 42 CNS neoplasms and 7 normal human brain samples, significantly higher levels (up to ten-fold higher) were observed in those tumors commonly associated with vascularity or cerebral edema (glioblastoma multiforme, hemangioblastoma, meningiomas). In those tumors not associated with increased vascularity and edema (pituitary adenomas and nonastrocytic gliomas), the levels of VPF/VEGF were not significantly different from those in normal brain. Cloning and sequencing of PCR-amplified GBM and normal brain cDNA demonstrated three forms of the VPF/VEGF coding region corresponding to mature polypeptides of 189, 165, and 121 amino acids, respectively. The relative abundance of the forms of VPF/VEGF mRNA was consistent in tumor and normal brain. Absorption of capillary permeability activity from human glioblastoma multiforme (GBM) cell conditioned medium and GBM cyst fluids by anti-VEGPF antibodies demonstrated that VEGPF is secreted by GBM cells and is present in sufficient quantities in vivo to induce vascular permeability.

We used Northern blot analysis and in situ hybridization histochemistry to establish that VPF/VEGF mRNA is expressed in the brain, kidney, liver, lung, and spleen of the adult rat. On Northern blots, the relative abundance of VPF/VEGF mRNA observed in these tissues was highest in the lung and lowest in the spleen. As determined by in situ hybridization, the patterns of VPF/VEGF expression are organ-specific.

The widespread expression and organ-specific distribution of VPF/VEGF mRNA in normal rat tissues, and the increased expression in human central nervous system tumors, suggest an extensive role for this factor in the physiology of both normal and tumor vasculature.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02674-08 SN
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Monoclonal Antibody-Toxin Conjugates for Tumor Therapy in vivo		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Richard J. Youle, Ph.D. Chief, Biochemistry Section, SNB, NINDS Dianne Newton, Ph.D. Staff Fellow, SNB, NINDS Susanna Rybak, Ph.D. Special Expert, SNB, NINDS Massimo Gadina, Ph.D. Special Volunteer, SNB, NINDS You-Neng Wu, Ph.D. Visiting Associate, SNB, NINDS		
COOPERATING UNITS (if any) Alfacell, Bloomfield, New Jersey		
LAB/BRANCH Surgical Neurology Branch, NINDS		
SECTION Biochemistry Section		
INSTITUTE AND LOCATION NINDS, National Institutes of Health, NINDS		
TOTAL STAFF YEARS: 4.0	PROFESSIONAL: 4.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Monoclonal antibodies</u> selectively bind tumor cell differentiating antigens in vitro and in vivo. Since natural effector mechanisms often do not mediate killing of monoclonal antibody bound cells so we have devised methods of linking extremely <u>toxic proteins</u> to the <u>antibodies</u> to selectively kill <u>tumor cells</u>. </p> <p> Two methods of coupling toxic proteins, (e.g., ricin to antibodies), have been used to kill antigen-positive cells in vitro. Ricin has two subunits: the A subunit blocks protein synthesis when in the cytosol, and the B subunit binds galactose groups on all cell surfaces but also facilitates the transport of ricin A chain to the cytosol. 1) Linkage of the ricin A chain to antibodies yields reagents with low nontarget toxicity but target cell toxicity too slow for in vivo applications; 2) and linkage of intact ricin to antibodies results in very potent target cell toxicity but the nontarget cell killing must be prevented by a ligand which blocks ricin B chain binding to cells. This has limited its application to in vitro situations where 100 mM lactose can block ricin binding. </p> <p> We have succeeded in developing several new approaches to apply <u>immunotoxins</u> in vivo. 1) Cloning toxins, then altering their structure at the gene level to decrease non target <u>cell toxicity</u>; 2) <u>intrathecal administration</u> of immunotoxins for therapy of <u>brain tumors</u> that kill 2-5 logs of <u>tumor cells</u> in <u>animal models</u>; 3) preparation of <u>genetically engineered immunotoxins</u> for clinical trials of human <u>brain tumor</u> patients; 4) prevention of an immune response against immunotoxin with anti-CD4 antibodies; 5) specific deletion of Purkinje cells in rats, guinea pigs, and rhesus monkeys; 6) use of human cytotoxic proteins such as RNase linked to antibodies to selectively target cells; and 7) understanding the mechanism of human RNase neurotoxins. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02697-08 SN

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protection of the Brain Against Injury by Ionizing Radiation with Pentobarbital

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D.

Chief, SNB, NINDS

Aytac Akbasak
Calvin Hawkins

Visiting Associate, SNB, NINDS
Bio Lab Technician, SNB, NINDS

COOPERATING UNITS (if any)

National Cancer Institute, Radiation Oncology Branch

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The efficacy of radiation therapy in the treatment of brain tumors is limited by the toxicity of ionizing radiation of the surrounding normal tissue.

In the rat model of cerebral radiation injury, pentobarbital has been shown to dose-dependently enhance overall survival. Evaluation of alternative barbiturates reveals that thiopental is of equivalent radioprotective value to pentobarbital. The rodent model of radiation injury does not parallel that of human injury. A primate model was designed to assess the role of pentobarbital in circumstances more applicable to the human situation. This ongoing study has thus far revealed the ability of pentobarbital to limit the toxicity of the radiation utilized. Neuroendocrinologic evaluation has revealed early dysfunction of thyroid-stimulating hormone, luteinizing hormone, and prolactin responses to stimulatory testing in the animals irradiated while anesthetized with ketamine. Significantly less abnormalities have occurred in the pentobarbital group.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02454-12 SN

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Human Pituitary Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward Oldfield, M.D.

Chief, SNB, NINDS

COOPERATING UNITS (if any)

Developmental Endocrinology Branch, NINDS

Diagnostic Radiology, CC

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, CNP

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We investigated venous sampling of the pituitary venous drainage to aid in the diagnosis and treatment of patients with Cushing's syndrome. Over 450 patients have now received bilateral simultaneous inferior petrosal sinus (IPS) sampling. The results indicate that 1) the procedure can be performed successfully in all patients with Cushing's syndrome (successful sampling has been performed in over 99% of the patients in whom it has been attempted); 2) the procedure distinguishes patients with ectopic ACTH secretion from those with pituitary adenomas with 100% accuracy; 3) IPS sampling successfully determines in which side of the pituitary gland microadenomas reside in patients with Cushing's disease with 70% accuracy; and 4) unilateral inferior petrosal sinus sampling, which is commonly used clinically, is frequently misleading.

Repeat transsphenoidal surgery is successful in eliminating the hypercortisolism of Cushing's disease in about 70% of patients. This therapy for patients with Cushing's disease after previous pituitary surgery had not previously been examined.

MRI scanning with and without gadolinium-EDTA was used to evaluate patients with Cushing's disease preoperatively. This technique permitted identification of the adenoma in about 55% of those patients with surgically proven microadenomas. Proper timing of the MRI after administration of gadolinium-EDTA was critical in the optimal use of the technique. Pituitary adenomas were detected in 15% of 100 normal subjects with MRI scanning with contrast.

ANNUAL REPORT

October 1, 1991 through September 30, 1992

**Clinical Neuroscience Branch
Clinical Neurosciences Program, DIR, NINDS
Irwin J. Kopin, M.D., Chief**

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ANNUAL REPORT

October 1, 1991 through September 30, 1992

Clinical Neuroscience Branch
Clinical Neurosciences Program, DIR
National Institute of Neurological Disorders and Stroke

Irwin J. Kopin, M.D., Chief

The Clinical Neuroscience Branch (CNB) research efforts are directed at determining the role of neurotransmitters and growth factors in regulating brain and peripheral autonomic development and function, elucidating abnormalities in transmitter metabolism, transport or receptor activation in diseases of the nervous system, and examining the molecular bases of alterations by therapeutic agents of these processes. At present, the Branch is comprised of four Sections, each conducting both independent research programs and collaborative projects involving investigators in other Sections or associated with organizations outside the Branch. The four Sections are: Molecular Genetics (Dr. Joan P. Schwartz); Aminergic Mechanisms (Dr. Irwin J. Kopin); Clinical Neurochemistry (Dr. David Goldstein); and Clinical Neuroparmacology (Dr. Irwin Kopin, Acting). During the past year, there have been several changes in personnel which provide an opportunity for redirecting some of the research programs of the Branch. Dr. Ronald Polinsky left to assume a position in the pharmaceutical industry and Drs. Eva Mezey and Saad Al-Damluji have joined the Branch as Visiting Scientist and Visiting Associate, respectively, to enhance molecular and integrative neurobiologic approaches to studies of aminergic mechanisms. The clinical research protocols on autonomic dysfunction are being transferred to the Clinical Neurochemistry Section, and the projects on familial Alzheimer's disease are being tapered to support only the collaborative studies initiated by Dr. Polinsky and Mrs. Linda Nee.

The research focus of the Molecular Genetics Section is on the expression and regulation of synthesis of neurotrophic factors and neuropeptides. Models of neurologic diseases in experimental animals and in tissue culture are used to analyze changes in the synthesis of content of neurotrophic factors in response to CNS injury or during recovery from toxic insult. Parkinsonian syndromes induced in mice or monkeys by systemic MPTP treatment, or in rats by intranigral administration of MPTP or 6-hydroxydopamine (6-OHDA) have been used to examine the formation by reactive astrocytes or by damaged neurons of trophic factors. In these models, expression of neurotrophic factors is determined by analysis of changes in specific mRNA content by blot hybridization, changes in peptide content by immunoassays, and appearance of neurotrophic activity by bioassay using chick dorsal root ganglia neurons or rat superior cervical ganglion or CNS neurons.

Primary cultures of astrocytes have proven to be particularly useful for studying the types of agents, including neurotransmitters, growth factors, and cytokines, which regulate the synthesis of neurotrophic factors, and for determining the molecular mechanisms for this regulation. Such astrocyte cultures are generally prepared from brains of newborn animals, but a technique for preparing and culturing astrocytes from any region of adult rat brain has been developed in our laboratory and is being used to compare the properties of adult astrocytes cultured from different regions of adult brain as well as differences in astrocytes between adult and newborn animals. It has recently been discovered that astrocytes cultured in the absence of other cells fail to continue their development; they are "frozen" at the stage at

which they were developed when the animals were sacrificed. This has allowed comparison of the reactive astrocytes present following neurotoxic lesions with normal astrocytes. In the past year, it has been demonstrated that nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are all expressed in astrocytes, but at levels which differ among brain regions and with developmental stage. Furthermore, synthesis of these factors can be stimulated by the neurotransmitter norepinephrine (NE), acting through the β -adrenergic receptors which are expressed in all astrocytes. Cytokines, such as interleukin-1 α or 1 β and γ -interferon, also increase neurotrophic factor expression, but effects are brain region specific. Since activated microglia in the brain can synthesize and release these cytokines, such agents may be important for inducing changes in neurotrophic factor expression following stress or after nervous system injury. In a collaborative study (with Dr. Michael Wells at SUNY, Stony Brook), it has been demonstrated that after lesioning of the sciatic nerve, NGF levels in the dorsal root ganglion are increased; preliminary data obtained by *in situ* hybridization and immunohistochemistry suggest that the increased NGF is synthesized in the Schwann cells. Schwann cell synthesis of NGF can be stimulated by IL-1, either by a direct effect of IL-1 or indirectly via another cytokine. Reactive astrocytes prepared from MPTP-lesioned mouse striatum synthesize 3-5 times more NGF mRNA and NGF than inactive astrocytes from undamaged brain. These increased amounts of NGF or a related neurotrophic factor appear to account for enhanced survival and neurite extension of cultured mesencephalic dopamine (DA) neurons by activated astrocytes than by astrocytes from nonlesioned striatum.

Some neuropeptide genes have been found to be expressed in astrocytes; such expression appears to be both gene- and brain region-specific. Astrocytes from virtually all brain regions express the proenkephalin gene and can synthesize and process the precursor of free enkephalin peptides. The pattern of processing appears to vary with the brain region; cerebellar astrocytes contain a C-terminally extended form of enkephalin. In contrast to proenkephalin mRNA, only cerebellar astrocytes have been found to express somatostatin mRNA and the corresponding peptide. Other opioid peptide precursors or substance P (SP) mRNA do not appear to be expressed by any astrocytes. Expression of proenkephalin and somatostatin appears to be regulated developmentally. Peak expression of somatostatin was found in astrocytes prepared from brains of rats taken between embryonic day 20 and postnatal day 3; much lower levels of expression was obtained using brains from 8-day-old animals, and levels were undetectable in cells prepared from adult animals. In contrast to somatostatin, astrocyte content of free enkephalins mRNA and unprocessed proenkephalin levels increased during development paralleling the levels found *in vivo*. Processing of proenkephalins of free enkephalins, however, appears to stop between day 3 and day 8. Thus, although both enkephalin and somatostatin contents of brain diminish between days 3 and 8, the decrement in somatostatin level occurs during gene transcription, whereas the decrement in enkephalin is attributed to lack of precursor peptide processing.

It has been suggested that during early CNS development enkephalins and/or somatostatin may act as astrocyte-derived trophic factors; recent data supports this possibility. Cerebellar granule cells in culture appear to develop their neuronal phenotype more rapidly in the presence of somatostatin. Maturation is reflected by increases in glutaminase (their neurotransmitter synthetic enzyme) and neurofilament protein. Although enkephalin has no apparent effect on cerebellar granule neurons, treatment of newborn rats with the opiate antagonist naltrexone for 1-2 weeks, at a dose sufficient to completely block opiate receptors, enhances the

rate of appearance of the neuronal phenotype. Naltrexone treatment of newborn animals also increases astrocyte content of NGF, along with changes in adenylate cyclase activity. Consistent with enkephalin inhibition of neurotrophic factor expression, reactive astrocytes prepared from MPTP-lesioned mouse striatum contain 10-fold less proenkephalin mRNA when NGF levels are increased. Thus, enkephalins may be involved in the down-regulation of neurotrophic factor expression during development. NGF content of hippocampal astrocytes, however, increases during development and is unaffected by naltrexone, perhaps as a result of brain region-specific regulation of NGF formation.

Neuropeptide expression has been examined after neuronal injury, with MPTP or 6-OHDA, after enhanced neuronal activity induced by electrical stimulation or by kindling (in the amygdala). Depletion of DA affects striatal enkephalinergic neurons differently than such neurons in the olfactory tubercle or in the cerebral cortex. Somatostatin levels in the cortex increase after selective NE depletion. Maprotilene (which blocks NE but not DA uptake) prevents both NE depletion induced by MPTP and the corresponding change in somatostatin. A single electrical stimulation increases proenkephalin and somatostatin mRNAs, whereas elevations of somatostatin mRNA in hippocampus and of proenkephalin mRNA in olfactory cortex are specific to kindling. These changes appear to occur in neurons, and to date there is no evidence for neuropeptide expression in astrocytes, but this may occur following specific forms of neuronal injury.

Recently a novel second/third messenger system, nitric oxide (NO), which is synthesized from arginine by NO synthetase, has been implicated in some models of neurotoxicity. Both NO synthetase mRNA and enzymatic activity have been demonstrated in cerebellar granule cells and astrocytes, and preliminary evidence suggests that MPTP may act in part by effects on astrocyte NO synthetase. Studies are currently underway to establish if NO is involved in MPTP-induced neurotoxicity and/or in neurotrophic factor responses to the injury.

The Aminergic Mechanisms Section conducts research directed toward quantifying and understanding regulation of amine metabolism, disposition, and receptor interactions and defining the integrative role of these neurotransmitters in responses to stress, during drug administration, and in association with neurologic or other disorders affecting aminergic function.

Studies have been performed in rats to examine responses and adaptation to different stressors: acute or repeated immobilization; acute and chronic exposure to cold; and acute hypoglycemia. In conscious rats, immobilization stress or even gentle handling rapidly elevates plasma catecholamine (CA) and metabolite levels, indicating rapid increases in CA release, reuptake and metabolism. After repeated intervals (2-hour) of immobilization (daily for 7 days), plasma NE levels and spillover are greater than after the first stress; the increase in plasma levels cannot be attributed to the slight reduction in clearance and metabolism of the CA.

After total adrenalectomy (but not after adrenal medullectomy) there is increased sympathetic activity reflected by an elevation of plasma levels of NE, dihydroxyphenylalanine (DOPA), and CA metabolites, during rest as well as in response to stress. There appears to be an inhibitory effect of the pituitary-adrenocortical system on sympathetic activity during stress.

Adrenal medullary tyrosine hydroxylase (TH) mRNA levels are increased 6-8-fold within 2 hours after initiation of the first interval of immobilization, whereas

dopamine- β -hydroxylase (DBH) and phenylethanolamine-N-methyl transferase (PNMT) mRNA levels are unchanged. However, within 3-6 hours after the first immobilization, mRNA levels for all three enzymes are significantly increased. One day after the first 2-hour interval of immobilization, concentrations of mRNA for TH, DBH and PNMT did not differ from the baseline. One day after the second daily 2-hour interval of immobilization, however, TH mRNA levels were again highly elevated but on this second day, DBH mRNA was elevated also. After 7 daily 2-hour intervals of immobilization, adrenal TH, DBH and PNMT mRNA levels were all greatly elevated immediately after the last stress interval. Thus, there appears to be a "memory" of the first immobilization stress such that the responses to subsequent intervals of stress are accelerated. The increases in adrenal TH mRNA are transcriptional since they are blocked completely by actinomycin D administration (1 mg/kg). Immobilization stress produces one of the most dramatic and rapid changes in expression of the CA biosynthetic enzyme genes seen.

During immobilization stress or even gentle handling, plasma DOPA levels and DOPA spillover into arterial blood increase rapidly, consistent with rapid activation of TH. The increase was abolished by ganglionic blockade with chlorisondamine, indicating that the DOPA responses were dependent upon postganglionic sympathetic activity. Since α -methyl-p-tyrosine administration prevents the increase in plasma DOPA levels, the increment may be assumed to reflect rapid increases in tyrosine hydroxylation.

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After total adrenalectomy (but not after adrenal medullectomy) there is increased sympathetic activity reflected by an elevation of plasma levels of NE, DOPA, and CA metabolites, during rest as well as in response to stress. There appears to be an inhibitory effect of the pituitary-adrenocortical system on sympathetic activity during stress.

Immobilization stress produces dramatic and rapid changes in expression of the CA biosynthetic enzyme genes. Adrenal medullary tyrosine hydroxylase (TH) mRNA levels are increased 6-8 fold within 2 hours after initiation of the first interval of immobilization, whereas dopamine- β -hydroxylase (DBH) and phenylethanolamine-N-methyl transferase (PNMT) mRNA levels are unchanged. However, within 3-6 hours after the first immobilization, mRNAs for all three enzymes are increased significantly. One day after the first 2-hour interval of immobilization, concentrations of mRNA for TH, DBH and PNMT did not differ from the baseline. One day after the second daily 2-hour interval of immobilization, however, TH mRNA levels were again highly elevated but in this case, DBH mRNA was elevated also. After 7 daily 2-hour intervals of immobilization, adrenal TH, DBH and PNMT mRNA levels were all greatly elevated immediately after the last stress interval. Thus, there appears to be a "memory" of the first immobilization so that the responses to subsequent intervals of stress are accelerated. The increases in adrenal TH mRNA are transcriptional since they are blocked completely by actinomycin D administration (1 mg/kg).

Immobilization stress or handling rapidly increased plasma DOPA levels and DOPA spillover into arterial blood consistent with activation of TH. The increase was abolished by ganglionic blockade with chlorisondamine, indicating that the DOPA responses were dependent upon postganglionic sympathoneural activity. Since methyl-p-tyrosine administration prevented the increase in plasma DOPA levels, the increment in plasma DOPA may be assumed to reflect rapid increases in tyrosine hydroxylation.

Effects of immobilization stress on the release of NE and its metabolites into the extracellular fluid of the paraventricular nucleus (PVN) of the hypothalamus or the central nucleus of the amygdala also have been studied in conscious rats using *in vivo* microdialysis. Concentrations of NE, dihydroxyphenylglycol (DHPG), methoxyhydroxyphenylglycol (MHPG), and dihydroxyphenylacetic acid (DOPAC), a DA metabolite, were measured before, during, and after the first and the seventh daily 2-hour interval of immobilization. The results indicate that acute immobilization increases the synthesis, release, and metabolism of NE in at least these two brain regions, and that repetition of immobilization decreases basal CA synthesis and noradrenergic turnover without inhibiting acute noradrenergic responses. Yohimbine increases NE release in the brain as well as in the peripheral tissues; this response is attenuated after chronic cortisol administration. Adrenalectomy augments stress-induced release of NE from the PVN.

Continuous exposure to cold (-3°C) or intermittent (interrupted by four hourly intervals at room temperature daily) exposure increases plasma NE and its metabolites. Although TH levels in brown adipose tissue increase with continuous cold exposure, adaptation to intermittent cold appears to be less effective than since TH levels are not increased when the cold exposure is interrupted. When adrenal medullary function is deficient, either continuous or intermittent cold appears to induce extraadrenal epinephrine synthesis. The hypothalamic-pituitary-thyroid axis is highly activated by continuous or intermittent cold exposure. It appears that hypothalamic-pituitary-adrenocortical system, which is greatly responsive to some stressors (e.g., immobilization), is not significantly affected by long-term cold exposure. Rats exposed to immobilization stress develop stomach ulcers. Similar ulcerations occur after treatment with cysteamine or with MPTP. To examine the molecular basis of such lesions, expression of receptors for histamine, gastrin, acetylcholine (muscarinic), and DA have been examined using *in situ* hybridization for the relevant mRNA. The numbers of plasmacytes and macrophages in the lamina propria of the stomach and duodenum increased markedly during stress, but not after cysteamine administration. These cells tend to migrate toward and enter the lumen of the GI tract. They were found to express mRNAs encoding a variety of receptors, as well as for TH. Surprisingly, many of these cells expressed mRNA encoding dopamine D₃, D₄ and D₅ receptors and a few expressed mRNA for histamine H₂, dopamine D₁ and D₂ receptors. After chronic immobilization, stress, but not after cysteamine, there was a striking increase in intensity of the mRNA expression. The gastrin receptor has also been demonstrated on 'immunocytes' in the lamina propria. The number of such cells increases dramatically as does the expression of message/cell following vagotomy and chronic immobilization stress. Dopamine D₅ receptors have been associated with macrophages, IgA negative plasma cells and a few IgG positive plasma cells, but not with mast cells.

A postsynaptic NE uptake system, which has the properties of the neuronal transporter (sodium-dependent, desipramine-sensitive, steroid-insensitive), has been found in the hypothalamus and in a GnRH-secreting cell line. After uptake by this mechanism, methylation and subsequent deamination limit accumulation of the

amine in the cells. Current plans are to compare the transporters in the cell line with known transporters using mRNA (in collaborative studies with investigators in the Laboratory of Cell Biology, NIMH) and if these indicate that this is a unique transporter, to then clone the transporter.

Research in the Clinical Neurochemistry Section has focused on developing and applying methods to assess the function of central and peripheral CA systems and the integration of these systems with other homeostatic mechanisms in health, stress, and disease.

Positron-emission tomographic (PET) scanning after systemic administration of 6-[¹⁸F]-fluorodopamine ([¹⁸F]-6FDA) has been used to visualize myocardial sympathetic innervation in normal volunteers. Assays of fluorodopamine and its metabolites in plasma and urine support the previous animal studies which indicate that visualization is the result of neuronal uptake and translocation into vesicles of [¹⁸F]-6FDA, its β -hydroxylation to [¹⁸F]-6F-norepinephrine. Pretreatment with desipramine, which inhibits neuronal uptake of NE and DA, markedly decreased uptake of [¹⁸F]-6F-DA-derived radioactivity in the human heart. This approach provides a noninvasive, *in vivo* means to examine cardiac sympathetic innervation and function in humans, and will be applied to studies of disorders of cardiac sympathetic regulation.

Direct measurement of skeletal muscle sympathetic neural activity makes possible diagnosis and monitoring effects of treatments in a variety of neurocardiologic disorders as well as studies of normal physiologic responses. Studies in normal volunteers and patients with neurocardiologic disorders are being directed at assessing the effects of pharmacologic and physiologic manipulations on the activity of the neurons. Thus far it has been demonstrated in normal subjects that such procedures can produce rapid changes in neuronal activity.

The first successful liquid chromatographic-electrochemical methods for plasma levels of normetanephrine (NMN) and metanephrine (MN) have been developed and validated. NMN and MN are the O-methylated derivatives of products of NE and epinephrine, respectively, and are indices of extraneuronal metabolism of these CA. Studies to date have shown that plasma NMN levels are above the normal range in all patients with pheochromocytomas; that small but detectable amounts of NMN are released in the human heart; and that blockade of oxidative deamination of CA increases plasma levels of metanephrines. Data about NMN levels are being applied in models using neurochemical approaches for examining aspects of regional sympathetic function in humans and to study the effects of inhibition of catechol-O-methyl transferase (COMT) in patients with Parkinson's disease.

In healthy humans, DA in urine was found to be derived mainly from renal uptake of plasma DOPA, and low-dose DOPA infusion evoked a marked natriuresis and diuresis. The role of the DOPA-DA system and therapeutic effects of DOPA are being explored in sodium-retaining disorders. Normal values for excretion rates of catechols in humans have been established. The excretion rate of DOPAC exceeds by far the excretion rates of all other catechols combined. Excretion rates of DOPA, DA, and DOPAC are all strongly positively correlated with sodium excretion in subjects on an *ad libitum* diet, consistent with compensatory activation of a DOPA-DA natriuretic system in response to increased dietary salt intake. Urinary DOPAC is derived partly from glomerular filtration of the metabolite and partly from metabolism of newly formed DA in the kidneys. In contrast, in humans all of the DA in urine appears to be derived from renal uptake and decarboxylation of plasma

DOPA. Infusion of L-DOPA at a very low rate (increasing plasma DOPA levels to only about 1/50 of those required to produce antiparkinsonian effects) evoked a marked natriuresis and diuresis. Patients with Cushing's disease, who have high circulating levels of cortisol, have low urinary DA excretion. Glucocorticoids therefore may inhibit renal DA production. Current studies are determining whether the ability to produce DA from plasma DOPA is impaired in salt-sensitive hypertension.

Changes in plasma levels of DOPA and DOPAC may reflect changes in the rate of CA synthesis. In children with neurodegeneration due to Menkes' disease, which is thought to be due to abnormal copper metabolism, neurochemical evidence indicated decreased activity of the copper-dependent enzyme, dopamine- β -hydroxylase (DBH). Plasma and cerebrospinal fluid NE levels were often normal in these patients; however, DOPA:DHPG and DOPAC:DHPG ratios were invariably increased. The elevated plasma DOPA and DOPAC levels suggest that increased TH activity can counter decreased DBH activity to maintain releasable NE stores.

In vivo microdialysis assessment of changes in extracellular fluid concentrations of CA and their metabolites in brain showed suggest that glucocorticoids inhibit α_2 -adrenoceptors on noradrenergic terminals in the PVN. Juvenile spontaneously hypertensive rats appear to have increased α_2 -adrenoceptor-mediated restraint of CA biosynthesis and NE release in the posterolateral hypothalamus and in the periphery. In other studies, it has been shown that glycine, administered locally in the striatum, enhances DA release, and that chronic inhibition of monoamine oxidase A increases exocytotic cerebrocortical NE release.

Corticotropin-releasing hormone (CRH) has been proposed to regulate activities of the body's main stress systems, the pituitary-adrenocortical system and the sympathoadrenal system, during stress. In rats, systemic or intracerebroventricular administration of a CRH antagonist failed to attenuate ACTH or epinephrine responses to insulin-induced hypoglycemia, and Lewis rats, which have deficient hypothalamic CRH responses to various stressors, had normal ACTH and epinephrine responses to insulin. The results failed to support the view that the ACTH and epinephrine responses to this metabolic stressor are regulated by increased CRH release in the brain. In humans, acute glucopenia induced by 2-deoxyglucose produced marked increases in ACTH and epinephrine levels; however, the responses of ACTH and epinephrine levels were unrelated, suggesting that the responses do not reflect a single central mechanism. Since plasma levels of the neuronal NE metabolite, dihydroxyphenylglycol (DHPG), and of DOPA and DOPAC failed to increase during 2-deoxyglucose-induced glucopenia, this stressor appears to stimulate adrenomedullary outflow much more than sympathoneural outflow, and the increased NE levels in this setting can be accounted for by profound adrenomedullary stimulation.

CA play key roles in homeostasis, stress, and disease. The sympathoadrenal system is one of the most powerful and rapidly-acting of the body's stress systems. Its two components - adrenomedullary hormonal and sympathetic neural - use epinephrine and NE as the main effector biochemicals. NE and its precursor CA, DA, are important central neurotransmitters, thought to be involved with movement, vigilance, memory, and distress. In the kidney, DA participates in sodium balance, and therefore regulation of extracellular fluid volume. The objectives of these research efforts have been to develop and apply methods to assess regional sympathoneural function, especially in the heart; and to understand how central and peripheral catecholaminergic systems are regulated and their function coordinated with those of other systems in stress and disease.

The Clinical Neuropharmacology Section had been involved in collaborative studies on the genetics of familial Alzheimer's disease and conducted studies on neuroendocrine and autonomic response deficits as well as experimental therapeutic approaches in orthostatic hypotension due to either multiple system atrophy (MAS) or peripheral autonomic failure (PAF). As indicated earlier, with the departure of Dr. Ronald Polinsky, research in this section is being refocused. The Section will continue to support the collaborative efforts involving genetic linkage and molecular studies to identify the etiology of Alzheimer's disease based on samples previously submitted to the Coriell Institute for Medical Research, Camden, New Jersey, for culturing of skin fibroblasts and peripheral blood lymphoblasts. This activity is supported through an intraagency agreement between the NINDS and NIA. A total of 23 families with an inheritance pattern consistent with autosomal dominant transmission have been included. Samples have been submitted from four categories of family members who undergo neurologic examination: affected; at-risk (first-degree), escapees, spouses. This is also part of an international collaboration organized and coordinated by our Section. Families from the U.S., Canada, Italy, France, and Germany are participating in this effort. The cultures continue to serve as a renewable source of DNA and cells for basic research on Alzheimer's disease.

Currently, two studies are being conducted in patients with PAF or MSA and in control subjects. The natriuretic and diuretic responses to graded doses of atrial natriuretic peptide (ANP) appear to be less than normal in patients with either type of autonomic failure, perhaps related to their greater hypotensive responses to ANP. Plasma CA and renin angiotensin assays are in progress. Patients with MSA appear to have enhanced sensitivity to hypercapnia-induced increase in ventilation - the relationship between increase in respiratory rate and tidal volume to plasma CO₂ levels is shifted to the right. The CA responses to hypercapnia are absent in both PAF and MSA individuals.

Simultaneous estimation of urinary excretion rates on plasma levels of homovanillic acid (HVA) (the major DA metabolite) and of MHPG and VMA (the major metabolites of NE) before and during administration of debrisoquin are used to better evaluate the contribution of brain DA metabolism to total body HVA formation. This method, which has been verified using animals treated with MPTP to eliminate brain dopaminergic neurons, is now being used to study DA metabolism in humans. Patients with early, untreated Parkinson's disease or with MSA appear to have low levels of HVA formation in brain, consistent with a deficit in available DA. Although it has been reported that brains of elderly persons have low levels of DA, the rate of formation of brain HVA does not appear to differ with age, suggesting that the turnover rate of brain DA increases with age to compensate for diminished DA content. The mechanism of the lowering of peripheral HVA production after debrisoquin administration may involve intraneuronal inhibition of monoamine oxidase, which not only diminishes HVA formation by blocking deamination, but also by directly inhibiting tyrosine hydroxylation (reflected by diminution of plasma DOPA levels), presumably as a result of elevated neuronal cytoplasmic CA affecting TH activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 NS 02630-08 CNB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical, Genetic and Biochemical Studies of Familial Alzheimer's Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.: Others:	R. J. Polinsky, M.D. L.E. Nee, M.S.W. J.A. Scott, M.D., Ph.D. J. Grafman, Ph.D.	Chief Social Science Analyst Senior Staff Fellow Psychologist CNS, CNB, NINDS OCD, CNP, NINDS CNS, CNB, NINDS CNU, MNB, NINDS
COOPERATING UNITS (if any) Genet. Unit, Toronto General, P. Hyslop; S. Ill. Univ. (R. Struble); Klinik Bavaria (P. Frommelt); Univ. of KY (J. Sikken); Univ. Göteborg, Sweden (A. McRae, A. Dahlström); Cedars-Sinai, CA (S. Pulst); J. Robbins, M.D. (NCI); M. Dalakas, M.D., NINDS; J. Kukus, Ph.D., (James Madison U.); Va, S. Wagner, (Salk Inst.)		
LAB/BRANCH Clinical Neuroscience Branch, CNP, DIR, NINDS		
SECTION Clinical Neuropharmacology Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither		
<input checked="" type="checkbox"/> (a1) Minors		
<input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) Alzheimer's disease (AD) is the most common cause of irreversible, chronic <u>dementia</u> . Although AD may be familial in only one-third of all cases, the main justification for studying autosomal dominant cases lies in the accuracy of diagnosis which may be inferred through postmortem examination of other affected family members. More than 270 members of 23 pedigrees with an autosomal dominant form of AD have had <u>skin fibroblast</u> and <u>peripheral blood lymphoblast</u> cultures established. These cultures serve as a renewable source of DNA and cell lines for genetic linkage, viability, and biochemical studies. <u>Recombinant DNA</u> technology has been applied to perform genetic linkage studies in these families with inherited AD. Approximately 75% of the amyloid precursor protein (APP) gene has been sequenced in the Canadian and Italian pedigrees. The β -amyloid peptide coding exons have been sequenced in 20 pedigrees. No mutations have been detected thus far. It appears that the Familial Alzheimer's disease locus and APP gene on <u>chromosome 21</u> reside at different locations in these pedigrees. Calcium transients induced in fibroblasts by serum and bradykinin are greater in control than in AD cells. Factors other than abnormal calcium metabolism or signal transduction may explain these observations. Longitudinal investigation of neurotransmitters/neuropeptides in CSF confirm our previous observations. The CSF level of <u>corticotropin releasing factor</u> (CRF) is normal in affected patients. Abnormally low plasma ACTH responses to arecoline suggest central cholinergic involvement in patients with familial AD. Only 25% of at-risk family members tested manifested a normal response to arecoline. Three at-risk subjects who later developed dementia had reductions in CSF MHPG, somatostatin, and regional cerebral glucose metabolism that preceded expression of the disease. Neurochemical and metabolic changes may have potential as biologic markers of AD.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02717-07 CNB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Preclinical Studies of Sympathoadrenal Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Graeme Eisenhofer, Ph.D., Senior Investigator, CNB

Others (CNB, NINDS): Inez Armando, Ph.D., Visiting Fellow, CNB
Richard Kvetnansky, Ph.D., Visiting Scientist, CNB
Ehud Grossman, Ph.D., Visiting Associate, CNB
Jacques Lenders, M.D., Visiting Associate, CNB
David S. Goldstein, M.D., Ph.D., Medical Officer, CNB
Irwin J. Kopin, M.D., Chief, CNB

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch, CNP

SECTION

Aminergic Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:

2.2

PROFESSIONAL:

1.7

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objectives of this project are to use animal models to develop and apply methods for examination of sympathoadrenal function, the ultimate goal of this being to extend these studies to the clinical arena. Tissue, plasma or urine samples are obtained before and during pharmacologic or physiologic manipulations and analyzed for concentrations of endogenous and exogenous radiolabelled norepinephrine and epinephrine, as well as their metabolites and precursors.

Increased plasma concentrations of dihydroxyphenylalanine (DOPA) during stimulation of sympathetic nervous outflow in rats and dogs were shown to be due to increased release from sympathetic nerves. Increased release of DOPA matched the increase in the turnover of transmitter stores indicating that changes in DOPA release provide an index of changes in the activity of tyrosine hydroxylase (TH).

Intravenous infusion of tritium-labelled catecholamines in the dog indicated that: (1) the lungs are an important organ for the release of norepinephrine and for the removal of circulating catecholamines; (2) removal is by neuronal uptake-like process, and (3) this process is more efficient for removal of norepinephrine than epinephrine.

In rabbits, examination of plasma concentrations of the deaminated intraneuronal metabolite of norepinephrine, dihydroxyphenylglycol, indicated that transmitter concentrations at sites of release were 3.4-fold greater than in plasma, and that this gradient was unaffected by sustained changes in sympathetic activity and was largely dependent on the efficiency of neuronal reuptake.

A liquid chromatographic method for the determination of plasma concentrations of the O-methylated catecholamine metabolites, normetanephrine and metanephrine, has been developed to examine the extraneuronal uptake and metabolism of catecholamines. Studies to date using this newly developed technique have concentrated on establishing the source and significance of plasma concentrations of metanephrines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02752-06 CNB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Synthesis and Expression of Neurotrophic Agents and Neuropeptides		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: Joan P. Schwartz, Ph.D., Chief, Molecular Genetics Section, CNB, NINDS		
Others: Nobuyoshi Nishiyama, Ph.D., Visiting Associate, CNB, NINDS Emil Viskupic, Ph.D., Visiting Associate, CNB, NINDS Dahlia Minc-Golomb, Ph.D., Visiting Fellow, CNB, NINDS Takayuki Taniwaki, M.D., Visiting Fellow, CNB, NINDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Neuroscience Branch, CNP		
SECTION Section on Molecular Genetics		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF-YEARS: 5.0	PROFESSIONAL: 5.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Evidence suggests that parallel biochemical and regulatory processes occur during normal development and following various forms of <u>CNS injury</u>. Among these areas of particular interest are: (1) identification of CNS neurotrophic factors; and (2) the analysis of the regulation of neuropeptide gene expression during development and in response to injury. Studies are underway to identify trophic factors produced in specific model systems, since recent evidence suggests that a family of nerve growth factors (NGF) exists, each specific for certain populations of neurons. An NGF-like factor increases in the cerebellum of the pcd mutant mouse as the Purkinje cells die out and astrocytes proliferate. MPTP-lesioned animals (both mice and monkeys) represent a Parkinsonian-like model in which changes in NGF and the related neurotrophic factors BDNF (brain-derived neurotrophic factor) and NT-3 (neurotrophin-3) are being examined at the level of mRNA, protein and biologic activity. Since astrocytes can synthesize NGF, primary cultures of astrocytes are being used to determine factors which regulate NGF gene transcription as well as to assess production of these other potential trophic factors. Reactive astrocytes are prepared from regions affected by the various injuries and their production of trophic factors compared to that of control astrocytes. Potential neurotrophic functions for the neuropeptides, enkephalin and somatostatin, in early CNS development are being explored in several model culture systems.</p> <p>At the same time, these injury models can be evaluated for changes in neuropeptide and/or neurotransmitter synthesis occurring in response to the lesions. One can derive an estimate of peptide turnover by combining measurements of the precursor mRNA, the precursor itself, and the peptide. Our studies have demonstrated that peptides are differentially regulated by such chronic drug treatments as reserpine, haloperidol, 6-hydroxydopamine or 5,7-dihydroxytryptamine. Work is in progress to determine the effects of CNS injury and <u>recovery</u>, including MPTP treatment, on various <u>neuropeptides</u> as well as such <u>neurotransmitter synthetic enzymes</u> as tyrosine hydroxylase and GAD, and the dopamine D2 receptor.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02839-02CNB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sympathoadrenal and Catecholaminergic Function in Health and Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: David S. Goldstein, M.D., Ph.D., Chief, Clinical Neurochemistry Section, CNB, NINDS; Others: Richard O. Cannon, III, M.D., CB, DIR, NHLBI; Anna Dekka-Starosta, M.D., Ph.D., Visiting Associate, CNB, NINDS; Graeme Eisenhofer, Ph.D., Visiting Associate, CNB, NINDS; John Finberg, Ph.D., Visiting Scientist, CNB, NINDS; Ehud Grossman, Ph.D., Sheba Medical Center, Tel Ha-Shomer, Israel; Courtney Holmes, Medical Technologist, CNB, DIR, NINDS; Harry R. Keiser, M.D., Chief, HE, DIR, NHLBI; Irwin J. Kopin, M.D., Chief, CNB, DIR, NINDS; Richard Kvetnansky, M.D., Ph.D., Visiting Scientist, CNB, NINDS; Jacques Lenders, M.D., Ph.D., Visiting Associate, CNB, NINDS; Karel Pacak, M.D., Visiting Fellow, DIR, NINDS; Arshad Quyyumi, M.D., CB, DIR, NHLBI; Efrat Wolfovitz, M.D., Visiting Fellow, CNB, DIR, NINDS; Gal Yadid, Ph.D., Visiting Fellow, CNB, DIR, NINDS

COOPERATING UNITS (if any)

NHLBI; and Sheba Medical Center, Tel Ha-Shomer, Israel

LAB/BRANCH

Clinical Neuroscience Branch, CNP

SECTION

Clinical Neurochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:

S

PROFESSIONAL:

S

OTHER:

0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our research has focused on developing and applying methods for assessing the function of central and peripheral catecholaminergic systems and the coordination of these systems with other homeostatic systems in health, stress, and disease. Findings this year include: (1) Positron-emission tomographic (PET) scanning after systemic administration of 6-[18F]fluorodopamine ([18F]-6F-DA) provided a non-invasive, *in vivo* means to examine cardiac sympathetic innervation and function in humans. This approach can be used to identify clinical disorders of cardiac sympathetic regulation. (2) Clinical microneurographic and allied methods were established to measure skeletal sympathoneural activity directly to diagnose and monitor effects of treatments in a variety of neurocardiologic disorders. (3) An assay method for plasma levels of metanephrines was developed and validated. High normetanephrine levels were noted in all pheochromocytoma patients tested. (4) In healthy humans, dopamine (DA) in urine was found to be derived mainly from renal uptake of plasma DOPA, and low-dose DOPA infusion evoked a marked natriuresis and diuresis. The role of the DOPA-DA system and therapeutic effects of DOPA are being explored in sodium-retaining disorders. (5) Plasma levels of DOPA and dihydroxyphenylacetic acid (DOPAC) reflect *in vivo* tyrosine hydroxylation, a key aspect of catecholaminergic function. (6) *In vivo* microdialysis assessments of changes in extracellular fluid concentrations of catecholamines and their metabolites in brain showed: (a) glucocorticoids inhibited the function of alpha₂-adrenoceptors on noradrenergic terminals in the paraventricular nucleus; (b) immobilization stress augmented norepinephrine (NE) release in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala; (c) juvenile spontaneously hypertensive rats had increased alpha₂-adrenoceptor-mediated restraint of catecholamine biosynthesis and NE release in the posterolateral hypothalamus and in the periphery, suggesting a basis for behavioral hyper-activity and hypertension in this rat strain; (d) locally administered glycine produced a net stimulatory effect on striatal DA release; and (e) chronic inhibition of monoamine oxidase A increased exocytotic cerebrocortical NE release. (7) Patterning of neuroendocrine stress responses during acute glucopenia in rats and humans refuted Selye's stress theory.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02870-01 CNB
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Brain Amines: Regulation and Function		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: I. J. Kopin, M.D., Chief, CNB, Director, DIR, NINDS Others (CNB, NINDS): G. Eisenhofer, Ph.D., Visiting Associate, CNB; K. Pacek, M.D., Visiting Fellow, CNB; Gal Yadid, Ph.D., Visiting Fellow, CNB; John Finberg, Ph.D., Visiting Associate, CNB; V. Weise, Chemist, CNB; J. Harvey-White, B.S., Technician, CNB; D. Goldstein, M.D., Ph.D., Medical Officer, CNB		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Neuroscience Branch, CNP		
SECTION Aminergic Mechanisms		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF-YEARS:	2.2	PROFESSIONAL:
	1.7	OTHER:
		0.5
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The main objectives of this project are to: (1) examine formation, release, metabolism and disposition of <u>brain biogenic amines</u> and their alterations after administration of drugs or toxin-induced models of human disease; (2) determine the physiologic role of biogenic amines in mediating responses to stress; and (3) develop methods that can be adapted to study of brain biogenic amine metabolism in humans.</p> <p><i>In vivo</i> microdialysis has been used to monitor levels of monoamines and their metabolites in extracellular fluid in various regions of the hypothalamus and in the basal ganglia. Receptors and transporters have been examined <i>in vitro</i> using cells from different regions of brain: in cell lines cultured from the hypothalamus; and in the gastrointestinal tract before and after immobilization (IMO) stress.</p> <p>During IMO, release of NE into the extracellular fluid (ECF) in regions of the hypothalamus and the amygdala is increased markedly. After repeated intervals of IMO, indices of NE synthesis and turnover that were not correlated with basal transmitter release in several areas. Yohimbine, an α_2-adrenoceptor antagonist, enhances release of NE, presumably by blocking presynaptic inhibition of release. This effect appears to be augmented in juvenile spontaneously hypertensive rats and to be attenuated after chronic cortisol treatment. Inhibition of MAO-B, but not MAO-A, gradually elevates ECF level of NE over a time interval of days, consistent with the gradual appearance of clinical efficacy of MAO inhibitors. Inhibition of MAO-A enhances the L-dopa-induced elevation of levels of dopamine in the ECF of the striatum. Using a new method for introducing agents directly into the region of the tip of a microdialysis probe, glycine was shown to stimulate release of dopamine from the striatum in a dose-dependent, strychnine-sensitive manner. In hypothalamic neurons, in culture and <i>in vivo</i>, there appears to be a novel desipramine-sensitive NE transporter which may be responsible for inactivation of the catecholamine.</p> <p>Microdialysis studies will be expanded to include amino acid and peptide neurotransmitters, additional stressors and hormones will be examined and molecular biology techniques will be applied to pursue the role of altered central catecholaminergic function in stress, during the development of hypertension in the SHR rat, and to characterize novel NE transporters.</p>		

ANNUAL REPORT

October 1, 1991 through September 30, 1992

**NEUROIMAGING BRANCH
Division of Intramural Research
Clinical Neurosciences Program
National Institute of Neurological Disorders and Stroke**

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ANNUAL REPORT

October 1, 1991 - September 30, 1992

NEUROIMAGING BRANCH
Division of Intramural Research
Clinical Neurosciences Program
National Institute of Neurological Disorders and Stroke

Giovanni Di Chiro, M.D., Chief

The Neuroimaging Branch conducts research to study, diagnose, and understand various pathologies of the central nervous system. Our techniques have advanced along with the improvements, or breakthroughs, in imaging methodology. Much of our present research involves Nuclear Magnetic Resonance, including localized spectroscopy of brain tumors, stroke, etc., studies to understand iron-generated MRI contrast, and others. Our Positron Emission Tomography imaging studies are also continuing, with a focus on the dopaminergic system and other areas.

RESEARCH SUMMARY

(1) Nuclear Magnetic Resonance (Imaging and Spectroscopy).

(a) Studies on localized proton spectroscopy (^1H -MRS) at 1.5 Tesla have been carried out in patients with brain tumors, stroke, epilepsy and neuropathic lipid storage diseases. Our emphasis has been on spectroscopic imaging. In tumor cases, correlation with PET-FDG studies has been particularly useful, allowing us to match regional glucose utilization with metabolite distribution maps. The most interesting finding has been the recognition of choline as a marker-metabolite for the actively proliferating tumoral parts. N-acetylaspartate (a neuronal marker) is reduced while the lactate presence fails to follow a consistent pattern in neoplastic tissues.

(b) Combined diffusion-perfusion imaging (using EPI-echo planar imaging) and ^1H MR spectroscopy have been performed in patients with stroke. Interesting correlations between diffusion changes and metabolite distribution (particularly lactate) in the cerebral tissues involved are beginning to emerge.

(c) Comparison of clinical MRI (with gadolinium-DTPA enhancement) results with those of PET-FDG, in a variety of abnormal conditions.

(d) Assessment by phase NMR imaging of pulsatile CSF flow and of spinal cord pulsation-motility.

(e) Analysis of the signal intensity changes in the striatum in MRI of patients affected by a variety of movement disorders.

(f) successful demonstration by MRI of selective basal ganglia damage following intracarotid injection of MPTP.

(g) In vitro studies of T1 and T2 of many chemicals of radiological interest, including paramagnetic ions and contrast agents, done with the variable-field T1-T2 analyzer, which was delivered in September 1991. Ferritin is of particular interest, because it is endogenous in some parts of the brain, and its effect on MRI is important, and may even have diagnostic implications.

(h) Imaging of monkeys of various ages at 0.15, 0.5, 1.5, 2.0 and 4.7 Tesla and post mortem measurement of iron content, to establish the effect of iron on MRI images in the normal brain at various ages.

(i) In vitro investigation of the relaxation times (T1, T2) of blood.

(j) The immediate and delayed effects of transient brain ischemia on diffusion-weighted images were studied using a cat model. We have observed that diffusion-weighted imaging is more sensitive to the effects of transient ischemia than conventional MRI. A second experimental (photochemical) model producing cerebral infarctions in rats is also being used.

(2) Positron Emission Tomography

(a) We have extended our comparison of PET studies in different disease processes with a variety of NMR imaging and spectroscopic techniques.

(b) A PET-FDG study of acoustic neuromas has been completed. A positive correlation has been found between tumoral glucose utilization rate and tumor aggressivity and recurrence propensity.

(c) A study is underway on glucose utilization (PET-FDG) of a relatively large group of patients with cerebral pyocytic astrocytomas.

(d) A collaborative project has been initiated with the Department of Neurosurgery of Georgetown University on PET-FDG studies in patients affected by neurofibromatosis type 2. These patients often harbor multiple intracranial tumors with different growth capabilities. Correlative studies on the glucose metabolism related to molecular genetics of these tumors are contemplated.

(e) PET-FDG studies on AIDS patients to differentiate opportunistic cerebral infections from lymphomas have been continued.

(f) A PET-FDG study on "dentate sparing" in crossed cerebellar diaschisis has been completed. A tentative explanation of the pathophysiology of "dentate sparing" has been offered.

(g) A complex investigation of steroid effects on cerebral metabolism, using [¹⁴C] deoxyglucose autoradiography in rats and PET-FDG in patients, is underway. Patients with Cushing's syndrome as well as cerebral glioma cases are included in this study.

(h) We have continued to follow, with PET-FDG, patients affected by familial (three families) Alzheimer's disease, as well as at-risk family members.

(i) We have continued our PET research on the dopaminergic system using FDG and 6-[¹⁸F] fluoro-L-dopa (6FD) in both animals (primate hemiparkinson model-MPTP) and human parkinsonians. Our lines of research have focused on: cerebral metabolism in human hemiparkinson; adrenal medullary autografts in human parkinson; caudate grafts in primates (normal and hemiparkinsonian); peripheral metabolism of 6FD and usage of enzyme inhibitors to increase cerebral L-dopa bioavailability.

(j) Single photon emission computed tomography (SPECT) studies using a new radiopharmaceutical (Tc-Ethyl-Cysteinate-Dimer-ECD) have been initiated in on-and off-medication for Parkinson's disease patients.

HONORS, AWARDS

"Ottorino Rossi Award" for outstanding contributions in the neurological sciences was conferred on Dr. G. Di Chiro by the University of Pavia, Italy. (Award included \$9,000 cash). Prize was presented during international meeting on "Functional and Therapeutic Neuroradiology" (June 1992), organized to honor Dr. Di Chiro.

Dr. Di Chiro was invited to give the keynote address at the 1992 annual clinical conference organized by the University of Texas, M.D. Anderson Cancer Center. Dr. Di Chiro was asked to speak on the diagnosis of brain tumors.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02315-15NB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Positron Emission Tomography

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Giovanni Di Chiro, M.D., Chief, NB, CNP, DIR, NINDS.

Others:

R.A. Brooks, Ph.D.	Staff Physicist	NB, NINDS
R. S. Miletich, M.D., Ph.D.	Sr. Staff Fellow	NB, NINDS
M. J. Fulham, M.D.	Visiting Associate	NB, NINDS
R. Raman, M.D.	Sr. Staff Fellow	NB, NINDS
M. J. Dietz, M.D.	Sr. Staff Fellow	NB, NINDS*

COOPERATING UNITS (if any)

CNB, NINDS; DIR, NINDS; NM, CC; BEIP, NCRR; LCM, NIMH; SNB, NINDS

LAB/BRANCH

Neuroimaging Branch, CNP, DIR

SECTIONS

Clinical Studies and Experimental PET

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

3.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
 ☐ (b) Human tissues
 ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Positron emission tomography (PET) is a nuclear medicine technique which allows us to obtain some anatomic data (e.g., axial, coronal or sagittal images of the brain) as well as dynamic functional data (such as regional cerebral glucose consumption rate). The unique property of PET is that it provides physiologic and pathophysiologic information not available with any other imaging procedure. Using a variety of radiopharmaceuticals as tracers, we have investigated with PET, brain tumors, movement disorders (Parkinson's disease, in particular), the dementias and cerebral involvement in AIDS. New information has been gathered, both in the basic and in the clinical (patient management) areas.

* Continued:

M. Quarantelli	Special Volunteer	NB, NINDS
C. Pierpaoli	Visiting Fellow	NB, NINDS
I. Linfante	Special Volunteer	NB, NINDS
K. Borbely	Visiting Associate	NB, NINDS
V. Sank	Special Expert	NB, NINDS
A. Brunetti	Visiting Fellow	NB, NINDS
E.H. Oldfield	Chief	SN, NINDS
C.V. Kufta, M.D.	Staff Physician	SN, NINDS
M. Hallett, M.D.	Clinical Director	CNP, NINDS
I.J. Kopin, M.D.	Director	DIR, NINDS
H.L. Shih, M.D.	DRRP Fellow	OIR, OD

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 NS 02073-19NB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nuclear Magnetic Resonance (Imaging and Spectroscopy)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Giovanni Di Chiro, M.D., Chief, NB, CNP, DIR, NINDS.

Others:

R.A. Brooks, Ph.D.	Staff Physicist	NB, NINDS
J.R. Alger, Ph.D.	Special Expert	NB, NINDS
M.J. Fulham, M.D.	Visiting Associate	NB, NINDS
R.S. Miletich, M.D., Ph.D.	Senior Staff Fellow	NB, NINDS
J. Vymazal, M.D.	CEEI Fellow	NB, NINDS

COOPERATING UNITS (if any)

DRD, CC; "In Vivo" NMR Research Center, BEIP; Division of Neuropathology of Case Western Reserve University, Medical School, Cleveland, OH

LAB/BRANCH

Neuroimaging Branch

SECTIONS

Clinical Studies and MR Spectroscopy

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

3.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our NMR imaging research is developing along the following lines: a) NMR spectroscopy (proton) in patients with tumors, stroke, epilepsy and lipid storage diseases; b) diffusion-perfusion imaging in patients with stroke and brain tumors; c) comparing clinical MRI imaging results with those of PET; d) analysis of iron accumulation in the basal ganglia of normal primates of various ages as well as in parkinsonian (MPTP) animals; e) analysis of the signal intensity from critical areas (basal ganglia) in patients affected by a variety of movement disorders; f) assessment of pulsatile CSF flow and of the "mobile" (normal) and "fixed" (pathologic) spinal cord; g) diffusion-perfusion imaging plus proton MR spectroscopy in experimental cerebral ischemia in cats and rats; h) in vitro studies of ferritin's NMR properties.

***Continued:**

A. Righini, M.D.		
M.J. Dietz, M.D.	Senior Staff Fellow	NB, NINDS
C. Pierpaoli, M.D.	Visiting Fellow	NB, NINDS
R. Raman, M.D.	Senior Staff Fellow	NB, NINDS
L.M. Levy, M.D., Ph.D.	Guest Researcher	NB, NINDS
A. Bizzi, M.D.	Visiting Fellow	NB, NINDS
A. Brunetti, M.D.	Visiting Fellow	NB, NINDS
H. Shih, M.D.	DRRP Fellow	OIR/OD
D. Le Bihan, M.D., Ph.D.	Visiting Scientist	DRD, CC
R. Turner, Ph.D.	Staff Physicist	CE, NHLBI
C. Moonen, Ph.D.	Staff Physicist	BEIP
J. Frank, M.D.	Medical Officer	DRD, CC
N. Patronas, M.D.	Staff Radiologist	DRD, CC

ANNUAL REPORT
October 1, 1991 through September 30, 1992

EPILEPSY RESEARCH BRANCH
Clinical Neurosciences Program, DIR
National Institute of Neurological Disorders and Stroke

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ANNUAL REPORT
October 1, 1991 through September 30, 1992

EPILEPSY RESEARCH BRANCH
Clinical Neurosciences Program, DIR
National Institute of Neurological Disorders and Stroke
Acting Chief, William H Theodore, M.D.

The Epilepsy Research Branch was established on December 6, 1991. It is divided into two sections in addition to the Office of the Chief. William H. Theodore, M.D., is Chief of the Clinical Epilepsy Section. Within the Clinical Epilepsy Section, Dr Susumu Sato is Chief of the Unit on Neurophysiology. Dr Michael Rogawski is Chief of the Neuronal Excitability Section.

The Branch conducts research on human and experimental epilepsy, including pathophysiology, the effect of seizures on cerebral function, and new approaches to diagnosis and treatment. It investigates the relationship between cerebral blood flow (CBF), energy metabolism, and functional activity in patients with epilepsy and normal controls. Normal and abnormal neuronal excitability are studied in several animal models, and electrophysiologic and neuropharmacologic aspects of epilepsy and antiepileptic drug (AED) action examined.

CLINICAL EPILEPSY SECTION

The CES studies patients with severe uncontrolled seizures using new techniques, in order to improve clinical control, as well as to elucidate the pathophysiology of epilepsy. The studies being carried out at this time focus on two main groups of patients: adults or children with complex partial seizures; and children with atonic / myoclonic seizures (the Lennox-Gastaut syndrome). Investigations are performed in collaboration with the EEG Laboratory, Neuronal Excitability Section, and Surgical Neurology Branch.

Neuroimaging techniques may be used to obtain both physiologic and anatomic data, and assist in the clinical evaluation of patients with severe seizures. Positron emission tomography (PET) uses intravenous injection of radioactive isotopes to determine regional rates of cerebral metabolism, blood flow, or tracer distribution. Magnetic resonance imaging (MRI) is being used to help elucidate the anatomic substrates of altered physiologic patterns revealed by PET. Digital image processing is used to co-register MRI and PET images. Initial attempts have been made to use nuclear magnetic resonance (NMR) spectroscopy to study biochemical parameters of human epileptic tissue *in vivo*. Single photon emission computed tomography (SPECT) is being used as an additional tool to study CBF. Clinical, EEG, and neuropsychological data, as well as ultrastructural and biochemical investigations of epileptic tissue removed at surgery, are correlated with function and structural results of imaging studies.

One of the major interests of the CES has been the use of neuroimaging techniques to study neuropharmacology. In clinical pharmacology research, it has always been much easier to derive pharmacokinetic than pharmacodynamic data. We have been taking advantage of the ability of PET to provide quantitative

information to study the effect of drugs on CBF and metabolism, as well as the distribution of putative neuroreceptor ligands in vivo.

Recent and ongoing studies:

Estimation of cerebral glucose metabolism using 18-fluoro 2-deoxyglucose PET (FDG-PET) showed that patients with uncontrolled complex partial seizures have regions of focal hypometabolism which correspond to electrographic localization of epileptic foci. The presence of PET hypometabolism predicts successful temporal lobectomy in these patients.

In the Lennox-Gastaut syndrome, a severe childhood epileptic encephalopathy, we found various patterns of neocortical hypometabolism with relative preservation of function in the basal ganglia and diencephalon, even when CT and MRI were normal. The results were in marked contrast to those obtained in patients with absence seizures, who had normal glucose metabolism. Distinct metabolic pathophysiologic processes were present in patients with distinct electroclinical epileptic syndromes.

We have used ^{15}O water to study CBF in patients with uncontrolled partial seizures. With this tracer it is possible to compare ictal and interictal states, as well as to compare blood flow with FDG scans performed in the same patients. Preliminary results indicate that there may be mismatches between blood flow and metabolism in epileptic tissue, and that glucose metabolism is reduced to a greater degree than blood flow. This may be an important marker for altered physiology in epileptic foci. Further studies are in progress, using improved PET techniques. Moreover, focal increases in blood flow have been found in patients who have had secondary generalized seizures during ^{15}O PET scans. These studies may help provide a means of localizing seizure onset.

We have been performing imaging studies of language organization in normal controls and patients with epilepsy. Using PET, activation of cerebral blood flow associated with word and object recognition, auditory comprehension, and phoneme, word, and sentence production are localized in the brain. Investigations are being conducted on classical conditioning and implicit and explicit memory performance. Data from subdural stimulation, PET, and MRI are integrated using digital image processing techniques. The combined stimulation and PET data allow us to study the relation between activation and disruption of cognitive activity, and to form more accurate concepts of the organization of cerebral function. These studies will elucidate the function of regions such as the basal temporal language area, which are of clinical importance when surgery for uncontrolled seizures is planned.

Several models of seizures, including kindled and postcardiac arrest audiosensitive rats, are being used to study patterns of neuronal damage and their relation to altered electrophysiology. Somatostatin (SS) neurons are selectively lost in the dentate hilus of patients with long standing temporal lobe epilepsy. These neurons are vulnerable to non-NMDA but not NMDA-mediated neurotoxicity in cell culture. NBQX, a non-NMDA antagonist, protected against loss of SS as well as Neuropeptide-Y (NPY) containing neurons, while MK-801 protected only against the former. Paired-pulse inhibition was lost in both experimental groups. SS and NPY immunoreactive neurons may not be responsible for this type of inhibition.

We studied the effect of AEDs on cerebral glucose metabolism. Phenobarbital depressed global LCMRglu by 30-40% while carbamazepine and phenytoin lowered glucose utilization by only 10-15%. These results are consistent with a possible difference between the mechanisms of action of the compounds. Phenobarbital interacts with the GABA-benzodiazepine receptor complex while phenytoin and carbamazepine appear to affect currents in active sodium channels. Moreover, other studies have shown that GABA agonists reduce glucose metabolism in experimental animals. Thus, the PET results may be of particular relevance to investigations into the mechanism of action of antiepileptic drugs. Moreover, they may provide evidence for explaining the difference in neuropsychologic toxicity, which is much greater for phenobarbital than for phenytoin or carbamazepine. Subsequently, valproic acid was found to reduce LCMRglc by 20-25%, suggesting that it may have a GABAergic effect at therapeutic doses in vivo.

Endogenous opiates have been implicated in the pathophysiology of epilepsy. 18F cyclofoxy, a naltrexone analogue, was used to image mu and kappa opiate receptors in patients with complex partial seizures. We found, in a few of the patients, increased opiate ligand binding ipsilateral to the epileptic focus, but no difference in the group as a whole. It is possible that reciprocal changes in Mu and Kappa receptors may obscure the true differences, and studies with more selective tracers are planned.

We have been evaluating the effect of drug withdrawal on seizure frequency, in order to assess the presence or absence of transient exacerbations, which could be distinguished from a simple loss of drug effect. This was clearly present in the case of phenobarbital, and carbamazepine, but absent for phenytoin. For carbamazepine, rate of discontinuation was significantly related to seizure frequency. Neuropsychiatric disorders such as panic were increased during drug withdrawal. These data are important for clinical practice. A physician wishing to withdraw a drug known to cause a transient exacerbation during taper may be more likely to persevere when seizures increase.

We have been conducting two double-blind placebo-controlled trials of felbamate, an experimental AED, in patients with complex partial seizures and the Lennox-Gastaut syndrome. Patients are entered into the complex partial seizure trial after they have been tapered off their other drugs for surgical monitoring. This process simplifies data collection and clinical screening for several potential trials.

Sphenoidal, multiple closely-spaced, and in some cases, subdural electrodes are used in the evaluation of potential surgical candidates, coupled with long-term video-EEG recording techniques. These techniques allow the acquisition of EEG data not available via conventional surface recordings. The data are correlated with PET and MRI to obtain the best possible presurgical localization of epileptic foci. Digital signal processing techniques based on the closely spaced electrode data in particular may allow surgery without invasive electrode monitoring in selected cases. A prospective evaluation of the relative value of invasive (subdural) and noninvasive methods of presurgical evaluation has been completed, and the data are being analyzed.

Magnetoencephalography (MEG) is a new approach to the problem of localizing abnormal cerebral potentials which may represent an epileptic focus. Initial studies suggest that MEG may provide more precise three-dimensional information than EEG, allowing detection and localization of epileptic foci in the depths of the brain, without the need for invasive procedures. Closely spaced electrode arrays are

applied for more precise mapping of surface abnormalities, and electrode position digitized. Both MEG and EEG data are then co-registered with MRI to compare anatomic localization using the two techniques. Source localization of spikes using various head models has been compared. Spherical models give inferior localization compared to more irregular, realistic approximations of head shape.

NEURONAL EXCITABILITY SECTION

During the 1991-92 fiscal year, the Neuronal Excitability Section (NES) under the direction of Dr. Rogawski, continued pharmacologic studies of NMDA- type glutamate receptor channels and ATP-sensitive and voltage-dependent K^+ channels using whole-cell voltage-clamp and single-channel recording techniques. In addition, a new direction was initiated on non-NMDA glutamate receptors. Complementary studies with drugs affecting ion channel activity were carried out in animal seizure and behavioral models. The aim of these studies was to explore new strategies for the rational development of AEDs based upon their interaction with ion channel systems that are critical to the regulation of CNS neuronal excitability and epileptogenesis.

Cellular Electropharmacology of NMDA Receptors

Excitatory neurotransmission mediated by NMDA receptors plays a critical role in epileptogenesis. Blockers of the NMDA receptor-associated ion channel, such as the dissociative anesthetics phencyclidine (PCP) and dizocilpine (MK-801), are powerful anticonvulsants. However, side effects, including ataxia and cognitive disturbances, limit the practical usefulness of these drugs in the treatment of seizure disorders. Researchers in the NES have identified several dissociative anesthetic analogs that are effective anticonvulsants in animal seizure models but have improved toxicity profiles compared to conventional dissociative anesthetic drugs. During the past year, work was continued on ADCl, a hybrid of dizocilpine and the widely used antiepileptic carbamazepine (CBZ), that is a low affinity uncompetitive NMDA antagonist. ADCl binds to and blocks the NMDA receptor-associated ionophore much more rapidly than do conventional dissociative anesthetic drugs, and it was proposed that the faster block may contribute to the lower toxicity of ADCl. In addition studies were completed showing that the purported polyamine site antagonist arcaine and the polyamine site inverse agonists DA10 and DA12 act as open channel blockers of the NMDA receptor associated ionophore. Finally, in collaboration with the Clinical Neuroscience Branch, NIMH, it was demonstrated that the neuroactive steroid pregnenolone sulfate (PS) augments NMDA receptor mediated increases in intracellular Ca^{2+} as measured by microspectrofluorimetry raising the possibility that PS or a related steroid acts as an endogenous agent contributing to the hyperexcitability in epilepsy.

Discovery of a Novel Noncompetitive Non-NMDA Antagonist

Among the most scientifically interesting results obtained by the NES during the past year was the finding of Drs. Donevan and Rogawski that the atypical benzodiazepine GYKI 52466 is a potent and highly selective noncompetitive antagonist of non-NMDA (AMPA/kainate) receptors. Although several selective competitive non-NMDA antagonists have been described previously, GYKI 52466 is

the first selective noncompetitive non-NMDA antagonist. Although structurally related to conventional benzodiazepines, GYKI 52466 does not affect GABA_A receptor-activated Cl⁻ currents, nor does it alter NMDA or metabotropic glutamate receptor responses. Consequently, GYKI 52466 will be an important tool to evaluate the function of non-NMDA receptor systems. In addition, the work demonstrates the existence of a novel recognition site for an atypical benzodiazepine on non-NMDA receptors and raises the possibility that there may be an endogenous ligand for this site. From the clinical perspective, noncompetitive non-NMDA antagonists may be superior to competitive non-NMDA antagonists in epilepsy therapy (or in the treatment of other neurological conditions associated with excessive glutamate release, such as brain ischemia) because the block they produce would not be overcome by high levels of glutamate. In fact, Drs. Yamaguchi and Rogawski have observed that GYKI 52466 is an effective anticonvulsant in the maximal electroshock seizure (MES) test and that it causes less neurologic toxicity than NBQX, a competitive non-NMDA antagonist.

Evaluation of Novel Noncompetitive NMDA Antagonists in Animal Seizure Models

During the past year, work continued on the characterization in animal seizure models of several low affinity noncompetitive NMDA antagonists. A study was completed in collaboration with the National Institute on Alcohol Abuse and Alcoholism demonstrating that ADCI is highly effective in preventing the occurrence of alcohol withdrawal seizures. In addition, studies with the enantiomers of ADCI demonstrated that the (+) isomer is approximately twice as potent an anticonvulsant (and has a somewhat greater therapeutic index) than the (-) isomer. ADCI has been licensed to an industrial collaborator (Neurogen Corporation, Branford, CT), and further preclinical work is continuing under a CRADA with the licensee.

K⁺ Channel Activator Drugs

Voltage-dependent K⁺ channels regulate neuronal excitability by repolarizing the neuronal membrane. Recently, Drs. Politi and Rogawski demonstrated that the benzopyran cromakalim can stimulate opening of ATP-sensitive K⁺ channels in cultured hippocampal neurons. During the present reporting period, studies were continued characterizing the unitary properties and regulation by intracellular factors of the cromakalim-activated channels. These channels, which have not previously been identified in neuronal cells, could play a role in protecting against brain ischemia, including that which may occur during prolonged seizures. Moreover, K⁺ channel activator drugs could be of use in the treatment of seizure disorders (and possibly also in protecting against seizure-induced brain damage).

Blockade of N-Type Ca²⁺ Channel in Hippocampal Neurons by Phencyclidine

A study was completed demonstrating that the dissociative anesthetic phencyclidine (PCP) produces a potent, use-dependent block of N-type voltage-dependent Ca²⁺ channels in acutely isolated CA1 hippocampal neurons. Since N-type Ca²⁺ channels regulate the release of neurotransmitters including glutamate, the unique anticonvulsant activity of PCP in certain seizure models could be due to its ability to prevent glutamate release as a result of N-type Ca²⁺ channel blockade. In fact, Drs. Yamaguchi, Coleman and Rogawski demonstrated that PCP is exceptionally potent

in protecting against the seizures and lethality produced by the K⁺ channel blocking agents 4-aminopyridine or dendrotoxin, whereas other dissociative anesthetic-like compounds that do not block Ca²⁺ channels were inactive. (In vitro studies have demonstrated that the epileptiform activity produced by K⁺ channel blockers is due to excessive release of glutamate acting on non-NMDA receptors.)

Cloned K⁺ Channel Genes Expressed in Fibroblasts

In view of the molecular heterogeneity of voltage-dependent ion channel proteins, it has become apparent that pharmacologic studies of ion channels will be much more useful if attention is focused on a molecularly defined population of channels. Cells bearing an homogeneous population of channels can be obtained by expressing cloned channel genes in cells that lack voltage-dependent ion channels, such as fibroblasts. Drs. Werkman and Rogawski have been exploring the physiologic and pharmacologic properties of a delayed rectifier-type voltage-dependent K⁺ channel expressed in a fibroblast cell line. During the present reporting period, studies characterizing the interaction of 4-aminopyridine and several K⁺ channel blocking peptides with the expressed K⁺ channel were completed. In addition, studies were initiated examining the regulation of the channel by intracellular factors, such as cAMP-dependent protein kinase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02236-17ERB*
PERIOD COVERED October 1, 1990 through September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Diagnostic and Therapeutic Reevaluation of Patients With Intractable Epilepsy		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	William Theodore, M.D. Chief, CES	ERBNINDS
Others:	Susumu Sato, M.D. Chief, EEG Lab	OCD NINDS
	William D Gaillard MD Medical Staff Fellow	ERB NINDS
	Susan Bookheimer PhD Staff Fellow	ERB NINDS
	Teresa Blaxton PhD Staff Fellow	ERB NINDS
	Laroy Penix MD Staff Fellow	ERB NINDS
COOPERATING UNITS (if any) EEG Laboratory, Office of The Clinical Director, NINDS		
LAB/BRANCH Epilepsy Research Branch, CNP, DIR		
SECTION Clinical Epilepsy Section		
INSTITUTE AND LOCATION NINDS, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS:	1.0	PROFESSIONAL: 1.0 OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The Clinical Epilepsy Section (CES) is using a multimodality approach to evaluate patients with severe <u>epilepsy</u>, including simultaneous <u>video and telemetered electroencephalographic (EEG) recording</u> of seizures, daily determinations of <u>antiepileptic drug serum concentrations</u>, <u>positron emission tomography (PET)</u>, <u>magnetic resonance imaging (MRI)</u>, and <u>magnetoencephalography (MEG)</u>. A specific seizure diagnosis is established allowing each patient to be assigned to an appropriate research protocol and therapy. PET uses radiolabelled tracers to measure cerebral glucose metabolism, blood flow, and neurotransmitter distribution. Focal <u>hypometabolism may underlie epileptogenic zones</u>. During seizures, increased glucose utilization and blood flow is found. In the <u>Lennox-Gastaut syndrome</u>, PET has revealed the existence of two separate metabolic patterns despite clinical seizure similarity. MRI may show small structural lesions underlying PET hypometabolism even when computed tomography (CT) is normal. Further studies will elucidate the relation of <u>metabolic and pathologic</u> changes. MEG may have the potential to accurately localize the subsurface origin of spikes. EEG provides little information on the <u>spatial distribution of epileptiform discharges</u> in cortical depths; MEG may be superior. Digital signal processing is being applied to data from multiple closely spaced electrode arrays. Comparison of invasive localization of epileptic foci using subdural electrodes and noninvasive evaluation is being performed. After surgery, patients are followed with serial clinical, neuropsychologic, and EEG evaluation. Children with partial seizures are followed with serial PET scans to assess the development of hypometabolism in the epileptic focus. The effect of the ketogenic diet is also being studied. Seizures in kindled and post cardiac arrest audio-sensitive rats are used to study patterns of neuronal damage and their relation to altered electrophysiology. Somatostatin neurons are selectively lost in the dentate hilus of patients with longstanding temporal lobe epilepsy. These neurons are vulnerable to non-NMDA but not NMDA mediated neurotoxicity in cell culture. NBQX, a non-NMDA antagonist, protected against loss of Somatostatin as well as NPY containing neurons, while MK-801 protected only against the former. Paired pulse inhibition was lost in both experimental groups. SS and NPY immunoreactive neurons may not be responsible for this type of inhibition.</p> <p>*Formerly in MNB; transferred to ERB in 12/91.</p>		
8-ERB/CNP/DIR		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02318-15ERB*

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Pharmacology of Antiepileptic Drugs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William H. Theodore, M.D.	Chief, CES	ERB	NINDS
Others:	Susumu Sato, M.D.	Chief, EEG LAB	OCD	NINDS
	William D Gaillard MD	Medical Staff Fellow	ERB	NINDS
	J Robert Flamini MD	Medical Staff Fellow	ERB	NINDS

COOPERATING UNITS (if any)

Office of The Clinical Director, NINDS

LAB. BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Clinical Epilepsy Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.2

PROFESSIONAL: 1.2

OTHER: 0

CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input checked="" type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Consistent with a possible difference between the mechanisms of action of the compounds, Phenobarbital (PB) depressed LCMRglu by 30-40%, valproic acid (VPA) by 20-25%, carbamazepine (CBZ) and phenytoin (PHT) by only 10-15%. PB interacts with the GABA-benzodiazepine receptor complex while PHT and CBZ appear to affect currents in active sodium channels. Moreover, other studies have shown that GABA agonists reduce glucose metabolism in experimental animals.

Endogenous opiates have been implicated in the pathophysiology of epilepsy. ¹⁸F cyclofoxy, a naltrexone analogue, was used to image mu and kappa opiate receptors in patients with complex partial seizures. We found, in a few of the patients, increased opiate ligand binding ipsilateral to the epileptic focus, but no difference in the group as a whole. It is possible that reciprocal changes in Mu and Kappa receptors may obscure the true differences, and studies with more selective tracers are planned.

We have been evaluating the effect of drug withdrawal on seizure frequency, in order to assess the presence or absence of transient exacerbations which could be distinguished from a simple loss of drug effect. This was clearly present in the case of PB and CBZ, but absent for PHT. For CBZ, rate of discontinuation was significantly related to seizure frequency. Neuropsychiatric disorders such as panic were increased during drug withdrawal. These data are important for clinical practice. A physician wishing to withdraw a drug known to cause a transient exacerbation during taper may be more likely to persevere when seizures increase.

We have been conducting two double blind placebo controlled trials of felbamate, an experimental antiepileptic drug, in patients with complex partial seizures and the Lennox-Gastaut syndrome, a severe childhood epileptic encephalopathy. Patients are entered into the complex partial seizure trial after they have been tapered off their other drugs for surgical monitoring. This process simplifies data collection and clinical screening for several potential trials.

* Formerly in MNB; transferred to ERB in 12/91

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1-0285801-ERB

PERIOD COVERED

October 1 1991 - September 30 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychological and Cognitive Studies in Epilepsy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: William Theodore, M.D. Chief, CES ERB NINDS

Others: William D Gaillard MD Medical Staff Fellow ERB NINDS

Susan Bookheimer PhD Staff Fellow ERB NINDS

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Medical Neurology Branch

LAB/BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Clinical Epilepsy Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL staff-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have been performing imaging studies of language organization in normal controls and patients with epilepsy. Using PET, activation of cerebral blood flow (CBF) associated with word and object recognition, auditory comprehension, and phoneme, word, and sentence production are localized in the brain. Investigations are being conducted on classical conditioning and implicit and explicit memory performance. Data from subdural stimulation, PET, and MRI are integrated using digital image processing techniques. The combined stimulation and PET data allow us to study the relation between activation and disruption of cognitive activity, and to form more accurate concepts of the organization of cerebral function. These studies will elucidate the function of regions such as the basal temporal language area, which are of clinical importance when surgery for uncontrolled seizures is planned. Digital signal processing techniques are used to confirm anatomic localization of functional mapping. Using surface fitting algorithms, PET, CT, MRI, and subdural electrode positions are aligned. In PET experiments, rest conditions are averaged and subtracted from activated conditions, in order to reveal regions of increased blood flow during task performance. Extensive speech deficits following inferior frontal gyrus (IFG) lesions have been explained by IFG involvement in either oromotor sequence planning, or syntactical and semantic processing. We recorded CBF in 6 normal volunteers using PET. Each performed 2 resting tasks and 4 speech tasks. All tasks led to increased CBF in orofacial primary cortex, including the supplementary speech area. But left IFG was active only when a prose passage was read. This suggests IFG plays a role in language processing, as well as planning oromotor sequences. We found that verbal and performance intelligence scores did not differ between patients with left and right temporal foci. Interictal discharges did not affect neuropsychological test scores. However, Boston Naming test scores, indicative of language function, were significantly lower in patients with left temporal seizure onset. These scores were not significantly lower after left temporal lobectomy.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01
NS 02732-06 ERB

PERIOD COVERED

October 1 1991- September 30 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacological Studies of Ion Channels in Cultured Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael A. Rogawski, M.D., Ph.D. NES, ERB, NINDS

Other:

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: Taco R. Werkman, Ph.D. NES, ERB, NINDS

: Sean Donevan, Ph.D. NES, ERB, NINDS

: Swaminathan Subramaniam, M.D., Ph.D. NES, ERB, NINDS

COOPERATING UNITS (if any)

Robert P. Irwin, M.D. PRAT Fellow, CNB, NIMH

Haruhiro Higashida, M.D. Kanazawa University, Japan

LAB/BRANCH

Epilepsy Research Branch

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL staff-YEARS:

4.5

PROFESSIONAL:

4.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Whole-cell voltage-clamp and single channel recording techniques were used to study drug interactions with voltage-dependent K⁺ channels and N-methyl-D-aspartate (NMDA) and non-NMDA receptor coupled cation channels in cultured hippocampal neurons and fibroblasts transfected with K⁺ channel genes. Work was focused in five areas: (i) intracellular regulation of ATP-sensitive K⁺ channels in CNS neurons, (ii) drug effects and intracellular regulation of molecularly defined K⁺ channel proteins in fibroblast cells transfected with K⁺ channel genes, (iii) kinetic analysis of low affinity noncompetitive NMDA antagonists, (iv) block of NMDA-activated cation channels by ligands purported to interact with the polyamine modulatory site, and (v) neurosteroid modulation of NMDA receptor responses. Cromakalim activates a K⁺ current in cultured hippocampal neurons. In inside-out patch recordings we demonstrated that the cromakalim-activated K⁺ channels can be regulated by intracellular ATP and ADP. Similar agents have potential as anticonvulsants, and in the treatment of brain ischemia. The blocking effects of 4-aminopyridine (4-AP) and the peptides charybdotoxin, dendrotoxin and mast cell degranulating peptide (MCDP) were investigated on the NGK1 voltage-dependent K⁺ channel (Kv1.2) expressed in fibroblast cells. The potent K⁺ channel blocking activity of 4-AP and the peptides may contribute to their powerful convulsant activity. Protein kinase A may regulate the Kv1.2 K⁺ channel. The novel anticonvulsant ADCI (5-aminocarbonyl-5H-dibenzof[a,d]cyclohepten-5,10-imine), a noncompetitive NMDA antagonist structurally related to the dissociative anesthetic dizocilpine and to carbamazepine. Unlike other dissociative anesthetics which cause motor toxicity at low doses, ADCI protects against seizures in animal models at doses that fail to cause motor impairment. In kinetic studies using whole cell voltage clamp techniques in cultured hippocampal neurons, we found the more favorable toxicity profile of ADCI may relate to its ability to block NMDA responses more rapidly than does dizocilpine. Arcaine, a putative competitive antagonist at the polyamine site of the NMDA receptor, and 1,10-diaminododecane and 1,12-diaminododecane, putative inverse agonists at the polyamine site, were found to produce an open channel block of the NMDA receptor, thus complicating the interpretation of their actions at the polyamine site. The muscle relaxant GYKI 52466 [1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine HCl] is a potent antagonist of non-NMDA-type glutamate receptor responses in cultured hippocampal neurons.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02733-06ERB*

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Excitability Properties of Enzymatically Dissociated CNS Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael A. Rogawski, M.D., Ph.D. Chief, NES, ERB, NINDS

Others: J.M.H. French-Mullen, Ph.D. ICI Americas, Wilmington, DE

COOPERATING UNITS (if any)

LAB/BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL staff-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The dissociative anesthetic phencyclidine (PCP) is a powerful anticonvulsant in a variety of animal seizure models. This activity is in part due to PCP's action as a noncompetitive (open channel) NMDA antagonist. However, PCP is an effective anticonvulsant in certain seizure models where other NMDA antagonists (such as dizocilpine) are inactive. Moreover, PCP produces a variety of side effects, some of which are not shared by other NMDA receptor antagonists. In whole cell voltage-clamp recordings, PCP was a potent blocker of N-type Ca^{2+} channels in acutely isolated guinea-pig CA1 hippocampal neurons. The block appeared to occur by binding of the drug to an activated state of the Ca^{2+} channel that is distinct from the open state. This use-dependent block of Ca^{2+} channels may contribute to PCP's anticonvulsant activity in some seizure models and may account for certain of PCP's unique behavioral actions, particularly those not shared by dissociative anesthetic-like compounds such as ketamine and dizocilpine which were only weak Ca^{2+} channel antagonists.

*Formerly in MNB; transferred to ERB 12/91

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01

NS 02772-05ERB*

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Uncompetitive NMDA Antagonists as Anticonvulsants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael A. Rogawski, M.D., Ph.D.	Chief, NES	ERB	NINDS
Others:	Shun-ichi Yamaguchi, Ph.D.	Psychologist, NES	ERB	NINDS
	Kenner C. Rice, Ph.D.	Chief, Lab of Medicinal Chemistry		NIDDK
	Izyaslav Lapin MD	Visiting Scientist	ERB	NINDS
	Brian R. de Costa, Ph.D.	Laboratory of Medicinal Chemistry,		NIDDK

COOPERATING UNITS (if any)

Neurogen Corporation, Branford, CT; Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN

LAB/BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL staff-YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

ADCI (5-aminocarbonyl-5H-dibenzo[a,d]cyclohepten-5,10-imine), a low affinity noncompetitive NMDA antagonist, was resolved into its optical enantiomers. (+)-ADCI was approximately twice as potent an anticonvulsant in the maximal electroshock (MES) test as (-)-ADCI and had a somewhat higher therapeutic index, suggesting that the (+) enantiomer may be more appropriate for further clinical development than the racemate. (±)-ADCI was highly effective in preventing the occurrence of seizures and tremulousness in mice made physically dependent upon ethanol. Thus, ADCI may be therapeutically useful for treating ethanol withdrawal seizures and other aspects of the ethanol withdrawal syndrome. The ability of various anticonvulsant drugs to protect against seizures produced by K⁺ channel blocking agents was evaluated in mice. Seizures were induced by intraperitoneal 4-aminopyridine (4-AP) or intraventricular dendrotoxin, a K⁺ channel blocking peptide. Phenytoin, carbamazepine and other phenytoin-like anticonvulsant drugs were effective in these models, but many other anticonvulsant drugs were inactive. ADCI was also highly effective in protecting against both 4-AP and dendrotoxin seizures. The muscarinic antagonists atropine and scopolamine were found to attenuate the increased locomotor activity produced by the noncompetitive NMDA antagonist dizocilpine. Muscarinic blockade is a potential strategy for preventing the adverse effects of NMDA antagonists. A series of 4-amino-(N-1-arylalkyl)-benzamides were evaluated for anticonvulsant activity in the mouse MES test. 4-Amino-N-(1-phenyl)cyclohexyl-benzamide was found to have a particularly favorable therapeutic index.

*Formerly in MNB; transferred to ERB 12/91



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